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ON OF THE RECORDING

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

OF A CHANGE

BILL, Kevin
AstraZeneca UK Limited
Global Intellectual Property
Mereside
Alderley Park
Macclesfield, Cheshire SK10 4T0
ROYAUME-UNI

	Macciestield, Cheshire SK10 41G			
Date of mailing (day/month/year)	7 ROY	ROYAUME-UNI		
09 May 2000 (09.05.00)	[]·			
03 May 2000 (03.03.00)			<u> </u>	
Applicant's or agent's file reference		7 · · · · · · · · · · · · · · · · · · ·		
PHM 70421/WO		IMPORTANT NO	TIFICATION	
11110170421700				
International application No.	Internatio	nal filing date (day/month/	(vear)	
PCT/GB99/03648		lovember 1999 (04.11		
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1. The following indications appeared on record concerning:	_		•	
X the applicant the inventor	the agen	nt the comm	non representative	
Name and Address	İ	State of Nationality	State of Residence	
ZENECA LIMITED	j	GB	GB	
15 Stanhope Gate London W1Y 6LN		Telephone No.		
United Kingdom				
omios Kingdom		Facsimile No.		
		i acsimile No.		
•	·			
	1	Teleprinter No.		
1 20 0 0 0				
2. The International Bureau hereby notifies the applicant that the	no following	ohanga haa haan sacasdaa	d conservations of	
X the person the name the add	ress	the nationality	the residence	
Name and Address		State of Nationality	State of Residence	
ASTRAZENECA UK LIMITED		GB	GB	
15 Stanhope Gate	- 1		1 08	
London W1Y 6LN		Telephone No.		
United Kingdom	0		,	
	1	Facsimile No.		
	}	Teleprinter No.		
		reteprinter 146.		
	`			
3. Further observations, if necessary:				
4. A copy of this notification has been sent to:		<u> </u>		
The state of the s	_			
X the receiving Office		the designated Office:	s concerned	
the International Searching Authority	Ē	the elected Offices co	ncerned	
	F			
the International Preliminary Examining Authority	L	other:		
The International Bureau of WIPO	Authorized	officer		

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland

S. De Michie!

Facsimile No.: (41-22) 740.14.35

Telephone No.: (41-22) 338 83,88



Copy-DMT

# NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

Date of mailing (day/month/year)

18 September 2000 (18.09.00)

From	the INTE	KNATIONAL BUI	(EAU
To:		R	<b>ECENIE</b>
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BILL, Kevin
AstraZeneca

Global Intellectual Property ASTRA 450000

P.O. Box 272, Mereside LUBAL INTELLECTUAL PROPERTY Alderley Park

Macclesfield, Cheshire SK10 4TG ROYAUME-UNI

Applicant's or agent's file reference PHM 70421/WO	IMPORTANT NOTIFICATION			
International application No.	International filing date (day/month/year)			
PCT/GB99/03648	04 November 1999 (04.11.99)			
The following indications appeared on record concerning:      The applicant the inventor	the agent the common representative			
Name and Address ASTRAZENECA UK LIMITED 15 Stanhope Gate	State of Nationality State of Residence GB GB			
London W1Y 6LN United Kingdom	Telephone No.			
	Facsimile No.			
	Teleprinter No.			
2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:  X the person the name the address the nationality the residence				
Name and Address	State of Nationality State of Residence SE SE			
ASTRAZENECA AB S-151 85 Södertälje Sweden	Telephone No.			
	Facsimile No.			
	Teleprinter No.			
3. Further observations, if necessary:				
4. A copy of this notification has been sent to:				
X the receiving Office	the designated Offices concerned			
the International Searching Authority  X the International Preliminary Examining Authority	X the elected Offices concerned other:			
The International Process of MIDO	Authorized officer L. LARRIE			
The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Christine Carrié			

Telephone No.: (41-22) 338.83.38

Form PCT/IB/306 (March 1994)

Facsimile No.: (41-22) 740.14.35

003530494



# REQUEST

For receiving Office use only
International Application No.
International Filing Date
Name of receiving Office and "PCT International Application"

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.    Name of receiving Office and "PCT International Application"		International Filing Date				
International application be processed according to the Patent Cooperation Treaty.    Applicant's or agent's file reference (if desired) (12 characters maximum)   PHM 70421/WO	The undersigned requests that the present	•				
Box No. I TITLE OF INVENTION  METHODS  Box No. II APPLICANT  Name and address: (Family name followed by given name; for a legal entity, full official designation, the address must include postal code and name of country. The country of the address indicated in this bax is the applicant is also inventor.  ZENECA Limited  15 Stanhope Gate  LONDON  GB-WIY 6LN  GB  State (that is, country) of nationality:  GB  This person is applicant  GB  State (that is, country) of nationality:  FR  This person is applicant  GB  State (that is, country) of nationality:  GB  State (that is, country) of nationality:  FR  This person is applicant only (that is check-box is marked, do not fill in below.)  FR  This person is applicant and/or (further) inventors are indicated on a continuation sheet.  Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE  The person identified below is hereby/has been appointed to act on behalf of the applicant(s) below the competent International Authorities as:  Name and address: (Family name followed by given name; for a legal entity, full difficial designation.  The person identified below is hereby/has been appointed to a	international application be processed					
Box No. I TITLE OF INVENTION  METHODS  Box No. II APPLICANT  Name and address: (Family name followed by given name: for a legal entity, full official designation: The address must include postal code and name of country. The country of the diddress indicated in this sor is the applicant of the part of the country of the diddress indicated below.)  ZENECA Limited  LONDON  GB-WIY 6LN  GB-WIY 6LN  GB-WIY 6LN  GB State (that is, country) of nationality.  Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)  Name and address: (Family name followed by given name: for a legal entity, full official designation in the applicant is state (that is, country) of residence if no State of residence is indicated below.)  GBEEN, Isabelle  Alderley Park  Macclesfield  Cheshire  GB-State (that is, country) of nationality:  This person is applicant and/or (further) inventors are indicated on a continuation sheet.  State (that is, country) of nationality:  GBEST-State (that is, country) of residence if no State of residence is indicated below.)  GBEST-State (that is, country) of nationality:  This person is applicant in the postal code and name of one state of the United States indicated below.)  GBEST-State (that is, country) of nationality:  This person is applicant only (If this check-box is market, do not full or below.)  GBEST-State (that is, country) of nationality:  The person indicated on a continuation sheet.  State (that is, country) of nationality:  The person indicated on a continuation sheet.  The person indicated on a continuation sheet.  State (that is, country) of residence: of America only o	according to the Patent Cooperation Treaty.					
METHODS  Box No. II APPLICANT  Name and address: (Family name followed by given name: for a legal unity, full official designation. The andress most market, proper and early and the states of the applicant state (family name followed by given name: for a legal unity, full official designation. The combined of the applicant state (family name followed by given name: for a legal unity, full official designation. Telephone No.  GB-WIY 6LN  GB						
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Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this box is the applicant of the full is, country) of residence if no State of residence is indicated below.)  ZENECA Limited 15 Stanthope Gate LONDON GB-W1Y GLN GB  State (that is, country) of nationality:  Telephone No. (01625) 583358 Telephone No.	METHODS					
This person is applicant   State (that is, country) of residence   Institute   State (that is, country)	Box No. II APPLICANT					
15 Stanhope Gate LONDON GB-W1Y 6LN GB  State (that is, country) of nationality: GB  This person is applicant for the purposes of: States states with include postal code and name of country. The country of the address must include postal code and name of country. The country of the dates indicated below.)  State (that is, country) of residence of the United States of America only the States indicated in of America only the Supplemental Box  Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)  Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)  Name and address: (Family name followed by given name; for a legal only, full official designation in this Box is the applicant is value (that is, country) of residence if no State of residence is indicated below.)  GBE-SK10 4TG GB  State (that is, country) of nationality: FR  State (that is, country) of residence: GB-SK10 4TG GB  State (that is, country) of nationality: FR  State (that is, country) of residence: GB-SK10 4TG GB  State (that is, country) of nationality: FR  State (that is, country) of residence: GB-SK10 4TG GB-SK10 4TG GB-SK10 4TG GB-SK10 4TG GB-SK10 4TG GB  State (that is, country) of nationality: FR  State (that is, country) of residence: GB-SK10 4TG GB-SK10	Box is the applicant's State (that is, country) of residence if no State of re	entity, full official designation. If the address indicated in this esidence is indicated below.)	This person is also inventor.			
LONDON GB-W1Y 6LN GB-W1 6L			Telephone No.			
State (that is, country) of nationality:    State (that is, country) of nationality:   State			(01625) 516173			
State (that is, country) of nationality:  GB  Teleprinter No. 669095/669388  State (that is, country) of residence  GB  This person is applicant for the purposes of:  This person is applicant state of the United States of America only the States indicated in of America only the Supplemental Box  This person is applicant for the purposes of:  This person is applicant of America only the States indicated in of America only the Supplemental Box  This person is applicant of State (that is, country) of residence is no State of residence is indicated below. If the States indicated in this Box is the applicant of State (that is, country) of residence is indicated below. If the States indicated below. If the United States of America only (If this check-box is marked, do not fill in below.)  State (that is, country) of nationality:  FR  State (that is, country) of residence:  GB  This person is applicant only (If this check-box is marked, do not fill in below.)  State (that is, country) of residence:  GB  This person is applicant only (If this check-box is marked, do not fill in below.)  The purpose of:  This person is applicant only (If this check-box is marked, do not fill in below.)  The purpose of:  The person is applicant only (If this check-box is marked, do not fill in below.)  The purpose of:  The person is applicant only (If this check-box is marked, do not fill in below.)  The person is applicant only (If this check-box is marked, do not fill in below.)  The person is applicant only (If this check-box is marked, do not fill in below.)  The person is applicant only (If this check-box is marked, do not fill in below.)  The person is applicant only (If this check-box is marked, do not fill in below.)  The person is applicant only (If this check-box is marked, do not fill in below.)  The person is applicant only (If this check-box is marked, do not fill in below.)  The person is applicant onl						
State (that is, country) of nationality: GB  State (that is, country) of residence GB  This person is applicant of the United States of America of America only the States indicated in for the purposes of:  Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)  Name and address: (Family name followed by given name: for a legal entity, full official designation in the Supplemental Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)  GREEN, Isabelle Alderley Park Macclesfield Cheshire GB-SK10 4TG GB  This person is applicant State (that is, country) of nationality: FR  State (that is, country) of residence: FR  State (that is, country) of residence: GB  This person is applicant only (If this check-box is marked, do not fill in below.)  State (that is, country) of nationality: FR  State (that is, country) of residence: GB  This person is applicant only (If this check-box is marked, do not fill in below.)  State (that is, country) of nationality: FR  State (that is, country) of residence: GB  This person is applicant only (If this check-box is marked, do not fill in below.)  State (that is, country) of nationality: FR  This person is applicant only (If this check-box is marked, do not fill in below.)  State (that is, country) of residence: GB  This person is applicant only (If this check-box is marked, do not fill in below.)  State (that is, country) of residence is indicated below.)  State (that is, country) of residence is indicated below.)  The country of the country of the address indicated below.)  State (that is, country) of residence is indicated below.)  This person is applicant only (If this check-box is marked, do not fill in below.)  This person is applicant only (If this check-box is marked, do not fill in below.)  The check of the states indicated in only of the address indicated below.)  The check of the states indicated below.)  The check of the states indicated below.)  This person is applicant only (If this check-box where no agent or co	GB					
This person is applicant for the purposes of:  States  Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)  Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the dadress indicated in this box is the applicant State (that is, country) of residence is no State of residence is indicated below.  State (that is, country) of nationality:  FR  This person is applicant and inventor  GB-SK10 4TG  GB  State (that is, country) of nationality:  FR  This person is applicant  This person is applicant and inventor  Inventor only (If this check-box is marked, do not fill in below.)  State (that is, country) of residence:  GB  This person is applicant  This person is applicant and inventor  Inventor only (If this check-box is marked, do not fill in below.)  This person is applicant  This person is applicant and inventor  The designated and designated and designated and inventor inventor only (If this check-box is marked, do not fill in below.)  The country of residence:  GB  This person is applicant  This person is applicant only (If this check-box is marked, do not fill in below.)  The designated and inventor  The designated and inventor only (If this check-box is marked, do not fill in below.)  The country of residence:  GB  This person is applicant  The United States except applicant inventors are indicated on a continuation sheet.  Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE  The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as:  Name and address:  (Family name followed by given name; for a legal entity, full official designation.  The address must include postal code and name of country. In the States indicated in the States indica			•			
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Name and address: (Family name followed by given name: for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)  GREEN, Isabelle Alderley Park Macclesfield Cheshire GB-SK10 4TG GB  State (that is, country) of nationality: FR  State (that is, country) of residence: GB  This person is applicant and inventor Cheshire GB-SK10 4TG GB  State (that is, country) of residence: GB  This person is applicant and inventor Cheshire GB-SK10 4TG GB  State (that is, country) of residence: GB  This person is applicant and inventor Inventor only (If this check-box is marked, do not fill in below.)  Fruther applicants and/or (further) inventors are indicated on a continuation sheet.  Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE  The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as:  Name and address:  (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)  BILL, Kevin Global Intellectual Property AstaZeneca PLC Mereside, Alderley Park Macclesfield, Cheshire, GB-SK10 4TG, GB  Address for correspondence: Mark this check-box where no agent or common representative is/has been appointed and the	The blaces indicated in					
GREEN, Isabelle Alderley Park Macclesfield Cheshire GB-SK10 4TG GB  State (that is, country) of nationality: FR  State (that is, country) of residence: GB This person is applicant for the purposes of: States and/or (further) inventors are indicated on a continuation sheet.  Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE  The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as:  Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)  BILL, Kevin Global Intellectual Property AstaZeneca PLC Mereside, Alderley Park Macclesfield, Cheshire, GB-SK10 4TG, GB  Adress for correspondence: Mark this check-box where no agent or common representative is/has been appointed and the	Box No. III FURTHER APPLICANT(S) AND/OR (FURT	HER) INVENTOR(S)				
GREEN, Isabelle Alderley Park Macclesfield Cheshire GB-SK10 4TG GB  State (that is, country) of nationality: FR  State (that is, country) of residence: GB This person is applicant for the purposes of: States and/or (further) inventors are indicated on a continuation sheet.  Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE  The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as:  Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)  BILL, Kevin Global Intellectual Property AstaZeneca PLC Mereside, Alderley Park Macclesfield, Cheshire, GB-SK10 4TG, GB  Adress for correspondence: Mark this check-box where no agent or common representative is/has been appointed and the	Name and address: (Family name followed by given name; for a legal e The address must include postal code and name of country. The country o	ntity, full official designation. f the address indicated in this	This person is:			
Alderley Park Macclesfield Cheshire GB-SK10 4TG GB  State (that is, country) of nationality: FR  State (that is, country) of residence: GB-SK10 4TG GB  This person is applicant for the purposes of:  all designated states except the United States of America only the States indicated in for the purposes of:  FR  This person is applicant for the purposes of:  FR  State (that is, country) of residence: GB  This person is applicant for the purposes of:  The States indicated in the States indicated in the States indicated in the States indicated in the Supplemental Box  The Duritor States  The United States The United States of America only The United States of America only The States indicated in t		sidence is indicated below.)	·			
Macclesfield Cheshire GB-SK10 4TG GB  State (that is, country) of nationality: FR  State (that is, country) of residence: GB This person is applicant of the purposes of: States and/or (further) inventors are indicated on a continuation sheet.  Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE  The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as:  I agent common representative  Telephone No. The address must include postal code and name of country.)  BILL, Kevin Global Intellectual Property AstaZeneca PLC Mereside, Alderley Park Macclesfield, Cheshire, GB-SK10 4TG, GB  Adress for correspondence: Mark this check-box where no agent or common representative is/has been appointed and the			applicant only			
GB-SK10 4TG GB  State (that is, country) of nationality: FR  State (that is, country) of residence: GB  This person is applicant for the purposes of:  This person is applicant states and/or (further) inventors are indicated on a continuation sheet.  Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE  The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as:  Name and address:  (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)  BILL, Kevin Global Intellectual Property AstaZeneca PLC Mereside, Alderley Park Macclesfield, Cheshire, GB-SK10 4TG, GB  Adress for correspondence: Mark this check-box where no agent or common representative is/has been appointed and the	Macclesfield		applicant and inventor			
State (that is, country) of nationality: FR  State (that is, country) of residence: GB  This person is applicant for the purposes of:  In the purposes of:  In the States indicated in the Supplemental Box  In the States indicated in the States indicated in the Supplemental Box  In the States indicated in the States indicated in the Supplemental Box  In the States indicated in			inventor only (If this shock have			
This person is applicant all designated the United States except for the purposes of:    States   all designated States except the United States of America of America only the Supplemental Box of America only the States indicated in the States indicated in the United States of America only the States indicated in			is marked, do not fill in below.)			
This person is applicant for the purposes of:  I all designated the United States except the United States of America only the States indicated in the Supplemental Box  Further applicants and/or (further) inventors are indicated on a continuation sheet.  Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE  The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as:  Name and address:  (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)  BILL, Kevin  Global Intellectual Property  AstaZeneca PLC  Mereside, Alderley Park  Macclesfield, Cheshire, GB-SK10 4TG, GB  Adress for correspondence: Mark this check-box where no agent or common representative is/has been appointed and the		State (that is, country)	of residence:			
Further applicants and/or (further) inventors are indicated on a continuation sheet.  Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE  The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as:  Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)  BILL, Kevin Global Intellectual Property AstaZeneca PLC Mereside, Alderley Park Macclesfield, Cheshire, GB-SK10 4TG, GB  Adress for correspondence: Mark this check-box where no agent or common representative is/has been appointed and the			GB			
Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE  The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as:  Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)  BILL, Kevin Global Intellectual Property AstaZeneca PLC Mereside, Alderley Park Macclesfield, Cheshire, GB-SK10 4TG, GB  Adress for correspondence: Mark this check-box where no agent or common representative is/has been appointed and the		d States except tates of America the	United States			
The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as:  Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)  BILL, Kevin Global Intellectual Property AstaZeneca PLC Mereside, Alderley Park Macclesfield, Cheshire, GB-SK10 4TG, GB  Adress for correspondence: Mark this check-box where no agent or common representative is/has been appointed and the	Further applicants and/or (further) inventors are indicated of	on a continuation sheet.				
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)  BILL, Kevin Global Intellectual Property AstaZeneca PLC Mereside, Alderley Park Macclesfield, Cheshire, GB-SK10 4TG, GB  Adress for correspondence: Mark this check-box where no agent or common representative is/has been appointed and the	Box No. IV AGENT OR COMMON REPRESENTATIVE	; OR ADDRESS FOR CO	ORRESPONDENCE			
The address must include postal code and name of country.)  BILL, Kevin Global Intellectual Property AstaZeneca PLC Mereside, Alderley Park Macclesfield, Cheshire, GB-SK10 4TG, GB  Adress for correspondence: Mark this check-box where no agent or common representative is/has been appointed and the	The person identified below is hereby/has been appointed to act of the applicant(s) before the competent International Authorities	n behalf as:	gent common representative			
Global Intellectual Property AstaZeneca PLC Mereside, Alderley Park Macclesfield, Cheshire, GB-SK10 4TG, GB  Teleprinter No. 669095/669388  Adress for correspondence: Mark this check-box where no agent or common representative is/has been appointed and the		ntity, full official designation. f country.)	Telephone No.			
AstaZeneca PLC Mereside, Alderley Park Macclesfield, Cheshire, GB-SK10 4TG, GB  Teleprinter No. 669095/669388  Adress for correspondence: Mark this check-box where no agent or common representative is/has been appointed and the			(01625) 512461			
Mereside, Alderley Park Macclesfield, Cheshire, GB-SK10 4TG, GB  Teleprinter No. 669095/669388  Adress for correspondence: Mark this check-box where no agent or common representative is/has been appointed and the			Facsimile No.			
Adress for correspondence: Mark this check-box where no agent or common representative is/has been appointed and the			(01625) 583358			
Adress for correspondence: Mark this check-box where no agent or common representative is/has been appointed and the	Macclesfield, Cheshire, GB-SK10 4TG, GB	•	Teleprinter No.			
Adress for correspondence: Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent			·			
and a second control of the c	Adress for correspondence: Mark this check-box where no space above is used instead to indicate a special address to w	Adress for correspondence: Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.				

		2	
Sheet	No.		

Continuation of Box No. III FURTHER APPLICANTS AND/OR (FURTHER) INVENTORS						
If none of the following sub-boxes is used, this sheet should not be included in the request.						
Name and address: (Family name followed by given name; for a let The address must include postal code and name of country. The count Box is the applicant's State (that is, country) of residence if no State (CHARLES, Andrew David Alderley Park Macclesfield Cheshire GB-SK10 4TG GB	gal entity, full official designation. try of the address indicated in this of residence is indicated below.)  This person is:  applicant only  x applicant and inventor  inventor only (If this check-box is marked, do not fill in below.)  State (that is, country) of residence:  GB					
This person is applicant all designated for the purposes of:	the United States except ed States of America only the States indicated in the Supplemental Box					
Name and address: (Family name followed by given name; for a leg The address must include postal code and name of country. The count Box is the applicant's State (that is, country) of residence if no State of	This person is:  applicant only  applicant and inventor  inventor only (If this check-box is marked, do not fill in below.)					
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#### (54) Title: METHODS FOR IDENTIFYING MODULATORS OF BS69 ACTIVITY

#### (57) Abstract

An endogenous human protein designated BS69 is identified as a new modulator of the Transforming Growth Factor- $\beta$  (TGF- $\beta$ ) cell signalling pathway. Methods are provided to identify compounds that interfere with the biological activity of BS69 on the TGF- $\beta$  cell signalling pathway. Such compounds have potential as therapeutic agents.

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## METHODS FOR IDENTIFYING MODULATORS OF BS69 ACTIVITY

A new modulator of the Transforming Growth Factor-β (TGF-β) cell signalling pathway is described, namely an endogenous human protein designated BS69. Methods are provided to identify compounds that interfere with the biological activity of BS69 on the TGF-β cell signalling pathway.

TGF-β itself regulates, *inter alia*, cell proliferation and differentiation, gene expression, embryonic development, extracellular matrix formation, haematopoiesis, apoptosis, wound healing, bone development and immune and inflammatory responses. The multiple effects of TGF-β lead to the medical need for both agonists and antagonists of its action. Jackson (Exp. Opin. Ther. Patents. (1998) 8(11):1479-1486) reviews key patents and scientific publications directed to modulation of the activity of TGF-β.

TGF-β cell signalling failure is implicated in a number of different tumour types and in the generation of human fibrotic disorders. Cell signal failure within other receptors in the TGF-β super family is implicated in other diseases such as arthritis, atherosclerosis, apoptosis, inflammation, wound healing and diabetic nephropathy. It is also known that administration of TGF-β helps prevent mucosistis and alopecia in patients undergoing chemotherapy or radiotherapy (PCT Publication No. 94/06459) and lowers resitance of multi-drug resitant malignant cells to chemotherapy (PCT Publication No. 92/13551).

TGF-β is one growth factor of a large super family which play broad roles in cell growth and differentiation in a variety of organisms. Examples of groups within the super family are the TGF-β set, activin/inhibin set, Mullerian inhibiting substance, glial cell line-derived neurotrophic factor and Bone Morphogenetic Proteins (BMPs).

Within the TGF-β subset the biological response to the growth factor is first initiated by binding of TGF-β to its respective receptor. The receptor is formed from two components TGF-β receptor I (TβR-I) and TGF-β receptor II (TβR-II) both of which contain cytoplasmic serine/threonine kinases and both of which are required for effective cell signalling (Wieser, R. et al., Mol. Cell. Biol. (1993) 13:7239-7247).

It was not until recently that the biological molecules involved in signal cell transduction of TGF-β were discovered. Through screening of genes in transgenic Drosophila expressing only partially active decapentaplegic (DPP), which is equivalent to BMP in

vertebrates, a new gene was found [Mothers against DPP(Mad)] which was able to restore the phenotype to the transgenic Drosophila expressing only partially active DPP protein (Sekelsky et al. Genetics (1995) 139:1347-1359). Analysis of the Mad gene sequence showed it to be closely homologous to the sma genes of *Caenorhabditis elegans* and the putative burnan tumour suppressor gene DPC4 (Deleted in Pancreatic Carcinoma). Such genes, of which several have now been identified, are now collectively referred to as Smad (Derynck, R. et al., Cell (1996) 87:173).

The Smad proteins constitute a unique signalling pathway which convey signals directly from TGF-β type receptors to the nucleus, where they modulate gene transcription.

There is close homology between many of the Smad proteins across species to such a degree that Smad proteins from one species may elicit a response in a different species. Many Smad proteins (Smad 1, 2, 3, 5 and others) are specific to the pathway associated with a particular receptor, others (Smad 4) act as a common mediator in different pathways, others (Smad 6 and 7) are inhibitory Smads that bind TGF-β receptor and block phosphorylation of the specific Smads. Current understanding of the TGF-β signalling pathway is briefly described below, a full review can be found in Heldin et al., (Nature (1997) 390: 465).

In brief, the mechanism of receptor cell signal transduction involves the following steps. For TGF-β signalling, the TβRI and II receptors are activated by autophosphorylation following TGF-β binding to the receptor complex. Smad 2 or 3 associate with the activated receptor complex and are themselves phosphoylated at a characteristic C-terminal Ser-Ser-X-Ser motif. After activation, the Smad 2/3 forms a stable complex with the common mediator Smad 4, which in turn translocates to the nucleus where it directly or indirectly modulates gene transcription.

As described above the TGF-β signalling pathway is implicated in a number of different diseases and as such this biological mechanism represents an attractive target for intervention in treating such diseases.

BS69 (Hateboer, R. et al., EMBO Journal (1995) 14(13):3159-3169 and PCT
Publication No. WO 97/00323) is described as being an adenovirus EI-A-associated protein
which inhibits EIA adenovirus gene transactivation. A later disclosure (Kurozumi, K. et al.,
30 (1998) 3(4):257-264) describes an alternatively spliced, and considerably shorter, form of

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BS69, which they call BRAM1 (BMP Receptor Associated Molecule 1), as being able to complex intracellularly with the BMP receptor.

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We have found through the use of protein hybridisation studies that the protein BS69 complexes with the Smad 2 and 3 proteins.

Whilst BMP is a member of the TGF-β superfamily of growth factors/receptor types it is known that BMP does not elicit signal transduction through Smad 2 or 3 proteins. In addition Kurozumi et al. found that full length BS69 did not complex with the BMP receptor and, therefore, no cellular function for the BS69 protein was described. Indeed, Kurozumi et al. remark "Therefore, the function of BRAM-1 may be different from that of BS69. At 10 present, the cellular function of BS69 is not known". The present inventors have discovered a new cellular mechanism of action for the BS69 protein.

We present as the first feature of the invention a method for the discovery of a modulator of BS69 activity, which method comprises contacting an assay system capable of presenting information on the effects of a chemotherapeutic agent on the activity of BS69 or a 15 derivative thereof with a potential chemotherapeutic agent under conditions in which BS69 is active in the absence of the potential chemotherapeutic agent and measuring the extent to which the potential chemotherapeutic agent is able to modulate the activity of BS69.

There is therefore provided, a method for identifying modulators of BS69 activity, which method comprises contacting an assay system, capable of presenting information on the 20 effects of a test compound on the activity of BS69 or a derivative thereof, with a test compound and measuring the activity of BS69.

Preferably BS69 activity may be described as the binding of BS69, or a fragment thereof to a human BS69 binding substrate. A "human BS69 binding substrate" is a protein endogenously expressed in human cells which is capable of having its biological function 25 modulated by binding of BS69. For the avoidance of doubt, adenovirus E1A protein is not a human BS69 binding substrate. Preferably the human BS69 binding substrate is selected from Smad 2, Smad 3, a complex of Smad 2 and Smad 4, and a complex of Smad 3 and Smad 4, or individual fragments thereof (herein after called BS69 binding substrate), more preferably the BS69 binding substrate is Smad 2 or Smad 3, or fragments thereof capable of 30 binding BS69. A "human BS69 binding substrate" may also be a nucleic acid to which BS69 or a protein complex comprising BS69 binds, such as a BS69 transcription factor dependent

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promoter or other regulatory elements which are affected directly or indirectly by binding of BS69. A preferred promoter element whose regulation is controlled in part by BS69 is plasminogen activator inhibitor-1 (PAI-1).

Thus according to a further aspect of the invention there is provided a method for the discovery of a modulator of BS69 activity, which method comprises contacting an assay system capable of presenting information on the effects of a chemotherapeutic agent on the binding of BS69 or a derivative thereof to a human BS69 binding substrate with a potential chemotherapeutic agent under conditions in which BS69 binds to the human BS69 binding substrate in the absence of the potential chemotherapeutic agent and measuring the extent to which the potential chemotherapeutic agent is able to modulate the activity of BS69.

According to a further aspect of the invention there is provided a method for the discovery of a modulator of BS69 activity, which method comprises contacting an assay system capable of presenting information on the effects of a chemotherapeutic agent on the binding of BS69 or a derivative thereof to a BS69 binding protein with a potential chemotherapeutic agent under conditions in which BS69 binds to the BS69 binding protein in the absence of the potential chemotherapeutic agent and measuring the extent to which the potential chemotherapeutic agent is able to modulate the activity of BS69.

According to a further aspect of the invention there is provided a method of screening for an agent useful in treating disorders characterised by an abnormality in a TGF-β signalling pathway, wherein said pathway involves an interaction between BS69 and a human BS69 binding partner, comprising screening potential agents for ability to disrupt or promote said interaction as an indication of a useful agent.

Potential chemotherapeutic agents which may be tested in the screen include those molecules, whether simple organic molecules, for example, of less than 2000 Daltons or larger biologic molecules, such as peptides, antibodies or DNA/RNA sequences, which may modulate the biology or pharmacology of BS69 activity, for instance by affecting the protein:protein binding of BS69 to a human BS69 binding substrate or by modulating the expression of DNA or RNA which encodes BS69. Suitable molecules include simple organic molecules, mimetics, nucleotide sequences, antibodies and any other molecules that modulate the activity of BS69. Chemotherapeutic agents/test compounds include both chemical and biological molecules.

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It will be appreciated that there are many assay systems which may be employed to perform the present invention. Examples of assay systems used to detect agents which may modulate the biological or pharmacological activity of BS69 are:

In vitro proximity assays such as a scintillation proximity assay (SPA), as described in 5 Udenfield et al., (Anal. Biochem. (1987) 161:494). In SPA derivatised microspheres which contain a scintillant and a fluorophore are used which attach through the derivatised group to a biological molecule of interest. When the biological molecule of interest binds a radiolabelled molecule then the proximity of the radiolabel to the scintillant causes increased emission of radiation signal and measurable increases in fluorophore excitation. In the present case, for 10 example, BS69 is bound to a scintillant/fluorophore containing microsphere through, for example, a streptavidin/biotin bridge, and a human BS69 binding substrate is radiolabelled or bound to a support with a radiolabel. Any potential chemotherapeutic agent which affects the way in which BS69 binds to the human BS69 binding substrate will affect the radiation emitted by the system. Alternatively instead of radiolabels and scintillants, fluorophore donor 15 and acceptor molecules may be used in what is called homogeneous time resolved fluorescence (HTRF), for example the acceptor fluorophore can be XL 665 and the donor fluorophore is europium (CIS Bio.). A further preferred feature of the invention is the invention as defined above wherein the assay system is a proximity assay, preferably SPA or HTRF.

In vitro cellular assay systems may be used. For example, a measurable output of BS69 activity could be detected when a reporter gene is placed under the control of the TGF-β signal transduction pathway. A stable cell is created which has a reporter gene under transcriptional control of the TGF-β pathway and which also expresses BS69, such a cellular assay may be prepared as described in US 5,436,128. Genes under TGF-β control which may be replaced by a reporter gene, for example by homozygous recombination, include plasminogen activator inhibitor-1, p15<sup>ink4b</sup>, and p<sup>WAFI</sup>, (Attisano et al., Biochemica et Biophysica Acta (1994) 1222:71-80; Hannon and Beach, Nature (1994) 371:257-261; and, Datto et al., J.Biol.Chem., (1995) 270:28623-28628). In this way, stimulation or inhibition of signal transduction results in stimulation or inhibition of reporter gene activity and potential test agents which interfere with BS69 activity may be detected. Suitable reporter genes include the β-galactosidase lac Z gene of E. coli (Casadaban et al., Meth. Enzymol. (1983)

100:293-308) or the firefly luciferase gene (de Wet et al., Proc. Nat. Acad. Sci. USA (1985) 82:7870-7873).

We present as a feature of the invention a method for the discovery of a modulator of BS69 activity, which method comprises contacting a potential chemotherapeutic agent with a cell comprising a reporter gene, expression of the reporter gene being under the control of the TGF-β signal pathway which is in turn under the control of BS69, a promoter which is activated by the TGF-β signal pathway and which has the gene encoding the reporter protein under its control, and determining modulation of BS69 by the potential chemotherapeutic agent by reference to any change in the expression of the reporter gene. Preferably the measurement of reporter gene expression is compared with a control cell construct wherein the reporter gene is under the control of the TGF-β signal pathway but in which BS69 is not expressed. The cell is preferably a mammalian cell, more preferably a stably transfected cell or cell line.

The promoter may be a naturally occurring promoter for TGF-β signalling, or it may be a synthetic promoter responsive to the TGF-β transduction pathway. Synthetic promoters would comprise one or more response elements to the signalling pathway, as well as elements such as a TATA box, required for correct transcription initiation. A preferred promoter is plasminogen activator inhibitor-1 (PAI-1).

The components of the TGF- $\beta$  signalling pathway may be endogenously expressed within the cells used in such assay, for example by the use of mammalian cell lines. Alternatively, components, such as heterologous receptors, may be expressed so that they couple to the TGF- $\beta$  signalling pathway. Also, an endogenous component may be removed, for example by gene deletion, and replaced with an exogenous protein which will restore the function of the pathway.

An alternative *in vitro* cellular system is the two-hybrid assay system. The two-hybrid system uses the ability of a pair of interacting proteins to bring a transcription activation domain into close proximity with a DNA-binding site that regulates the expression of a reporter gene. Commercially available systems such as the CLONTECH, Matchmaker<sup>TM</sup> systems and protocols may be used with the present invention. (*See also*, Mendelsohn, A.R., Brent, R., Curr. Op. Biotech., 5:482 (1994); Phizicky. E.M. and Fields, S., Microbiological Rev., 59(1):94 (1995); Yang, M., et al., Nucleic Acids Res., 23(7):1152 (1995); Fields, S. and

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Sternglanz, R., TIG, 10(8):286 (1994); US Patents 5,283,173 and 5,468,614). Two hybrid screening systems can be practised either with a positive readout or with a negative readout. Recently, some examples of "reverse" two-hybrid systems have been described. Leanna, Ž C.A. and Hannick, M. (Nucl. Acids Res. (1996) 17:3341-3347) use an output in which a gene 5 under the control of the two hybrid system is toxic in the presence of cycloheximide. Vidal, M., Brachmann, R., K., Fattaey, A., Harlow, E. and Boeke, J.D. (Proc. Natl Acad. Sci. U.S.A. (1996) 93:10315-10320) use the property of the URA3 gene product that it can be selected against by 5-fluoro-orotic acid. It is possible to test the ability of a potential chemotherapeutic agent to interfere with the binding of BS69 and a BS69 binding substrate, where BS69 is 10 expressed as a fusion protein to a part of a transcription factor, either the transcription activation domain or the DNA-binding site, and the human BS69 binding substrate is expressed as a fusion protein to the other part of the transcription factor. Such that, if hybridisation of the transcription factor is prevented from occurring by a chemotherapeutic agent then transcription of a reporter gene under transcriptional control of the transcription 15 factor is interrupted.

Several variations on the two hybrid system are known, and may be configured for use in the present invention. For example, a "tribrid" system has been described in which the two hybrid interaction will only occur if one component is phosphorylated by a kinase introduced into the cell (Osborne, M.A., Dalton, S. and Kochan, J.P. (1995) Bio/Technology 13, 1474-20 1478).

The two hybrid or tribrid systems can be adapted for use in yeast or, preferably, mammalian cells.

We present as a feature of the invention a method for the discovery of a modulator of BS69 activity, which method comprises contacting a potential chemotherapeutic agent with a cell comprising a transcription factor dependant promoter, a reporter gene under the control of the transcription factor dependant promoter, a fusion protein of BS69, or a human BS69 binding substrate, and a domain of a transcription factor which binds to the promoter and a second fusion protein of a human BS69 binding substrate, or BS69, and a domain of the transcription factor which activates transcription, wherein binding of BS69 to the human BS69 substrate causes the two domains of the transcription factor to become disposed to promote expression of the reporter gene, and determining modulation of BS69 activity by the

potential chemotherapeutic agent by reference to any change in the expression of the reporter gene. It will be apparent that in the above system one of the fusion proteins will have a BS69 binding component and the other will have a BS69 binding substrate component.

As described above, an alternative approach to the intervention of BS69 binding to a BS69 binding substrate is to affect gene transcription or gene translation in the cell and thus prevent BS69 protein production in the cell. A variety of points in these processes may be disrupted such as by interference by a chemotherapeutic agent in the binding of BS69 transcription factors to the upstream promoter sites or by a chemotherapeutic agent binding to the coding DNA or mRNA (such as anti-sense nucleotides) of BS69.

Assay methods which may be utilised in the performance of the above aspect of the invention include those disclosed in European Publication No. 0483249.

Compounds that modulate the expression of DNA or RNA encoding the BS69 polypeptide may be detected by a variety of assays. The assay may be a simple "yes/no" assay to determine whether there is a change in expression of a reporter gene. The assay may be made quantitative by comparing the expression or function of a test sample with the levels of expression or function in a standard sample. Systems in which transcription factors are used to stimulate a positive output, such as transcription of a reporter gene, are generally referred to as "one-hybrid systems" (Wang, M.M. and Reed, R.R. (1993) Nature 364:121-126). Using a transcription factor to stimulate a negative output (growth inhibition) may thus be referred to as a "reverse one-hybrid system" (Vidal et al, 1996, supra). Therefore, in an embodiment of the present invention, a reporter gene is placed under the control of a BS69 promoter.

In a further aspect of the invention we provide a heterologous cell wherein expression in the cell of a reporter gene is under the control of a BS69 transcription factor dependent promoter, and wherein expression of the transcription factor is inducible, whereby inhibition of gene transcription by the potential chemotherapeutic agent may be determined by reference to a lack of expression of the reporter gene.

In a further aspect of the invention we provide a cell or cell line comprising a reporter gene under the control of a BS69 transcription factor dependent promoter.

We also provide a method for identifying inhibitors of BS69 transcription which method comprises contacting a potential therapeutic agent with a cell or cell line as described

above and determining inhibition of BS69 transcription by the potential therapeutic agent by reference to a lack or reduced expression of the reporter gene.

A method for identifying modulators (activators or inhibitors) of BS69 transcription which method comprises contacting a cell or cell line as described above, said cell or cell line also supplied with exogenous or endogenous BS69, with a test compound and determining the effect on BS69 transcription by the test compound by reference to enhanced or reduced expression of the reporter gene.

In general, eukaryotic transcription factors consist of a DNA binding domain and a transcription activation domain (Ptashne (1988) Nature 335:683-689). Frequently these 10 factors are dimers. Thus there may be three interfaces at which interference with a chemotherapeutic agent may inhibit a transcription factor: the DNA:protein interface, the dimerisation interface, and the interface between the activation domain and the transcription apparatus (Peterson, M.G. and Baichwal, V.J. (1993) TibTech 11:11-18). To find inhibitors of the interaction of mammalian DNA binding protein with its binding site, a transcription 15 activation domain is fused to said DNA binding domain in order to make a transcription factor which functions in the cell type of interest. Conversely, if the interaction between an activation domain and the transcription machinery is of interest, a DNA binding domain may be fused to the activation domain of interest to yield a transcription factor. In such circumstances, it may also be desirable to express within the cell the protein which the 20 activation domain contacts. Generally, activation domains are believed to activate transcription through recruitment of the RNA polymerase holoenzyme (Ptashne, M. and Gann, A. (1997) Nature 386:569-577). This recruitment occurs through protein:protein interactions. Using genetic techniques it is possible to substitute components of the S. cerevisiae holoenzyme for mammalian homologues. In this way the protein:protein 25 interaction of interest may be reconstituted using components from the same species.

Reference to BS69 its polypeptide, DNA or RNA sequences include references to derivatives thereof. Derivative polypeptides, or DNA/RNA sequences, of BS69 include:

- i) allelic variations of BS69, in particular any single nucleotide polymorphism (SNP);
- ii) a fragment of BS69, i) or iii) capable of binding to BS69 binding substrate; and
- 30 iii) a mutant form of BS69, i) or ii),
  and, preferably, exclude BRAM1.

Particularly preferred polypeptide fragments are those which are at least 15 amino acid long and include wholly or at least partially the Smad binding domain of BS69. The Smad binding domain has been localised to between about amino acid 450 and the C-terminus of the BS69 protein (as 562) disclosed as SEQ ID No. 2 in PCT Publication No. WO 97/00323.

5 Thus, preferred fragments or polypeptides of BS69 include this region. In WO 97/00323 the E1A binding domain was localised to between amino acids 412 and 532. We have found that the Smad2 binding domain is contained within amino acids 443-562. It is evident that the area of BS69 that mediates interaction with E1A also mediates interaction with Smad2. It is likely therefore that the domain lies within the region defined by amino acids 443 and 512.

10 Particularly preferred fragments for use in the invention include the BS69 polypeptide sequence from amino acids 450-562 or 443-562 or 443-512 or 450-512.

For the purposes of this application the nucleic acid and amino acid sequence of BS69 referred to herein are disclosed in PCT Publication No. WO 97/00323 SEQ ID NO:1 and 2.

Allelic variations or SNPs in the BS69 DNA sequence may be detected by alteration in the pattern of restriction fragment length polymorphisms capable of hybridising to SEQ ID NO:1 of WO 97/00323 or by the inability of allele-specific oligonucleotide probes to specifically hybridise to SEQ ID NO:1 of WO 97/00323 under appropriate conditions. BS69 SNPs can also be determined by nucleic acid sequencing.

It will be readily appreciated by the skilled reader that as a result of the degeneracy of the genetic code, a multitude of sequences some having minimal homology (sequence identity) to any naturally occurring gene for BS69, may be produced and found to have utility in the present invention. Thus, the invention contemplates each and every possible variation of nucleotide sequence based on possible codon choices coding for the same amino acid.

Monospecific antibodies to BS69 may be purified from mammalian antisera

25 containing antibodies reactive against the polypeptide or are prepared as monoclonal antibodies reactive with the BS69 using the technique of Kohler and Milstein, (Nature, (1975) 256:495). Mono-specific antibody as used herein is defined as a single antibody species or multiple antibody species with homogenous binding characteristics for BS69. Homogenous binding as used herein refers to the ability of the antibody species to bind to a specific antigen or epitope. BS69 specific antibodies are raised by immunizing animals such as mice, rats, guinea pigs, rabbits, goats, horses and the like, with rabbits being preferred, with an

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appropriate concentration of BS69 either with or without an immune adjuvant and optionally conjugated to a carrier protein such as albumin.

Further features of the invention include:

A method of treatment of a patient in need of such treatment for a condition which is mediated by the biological or pharmacological activity of BS69 on a human BS69 binding substrate, comprising administration of a polypeptide substantially as depicted in WO97/00323 SEQ ID NO:2 or a pharmacologically active fragment thereof.

A method of treatment of a patient in need of such treatment for a condition which is mediated by the biological activity of BS69 on a human BS69 binding substrate, comprising administration of a nucleic acid substantially as depicted in WO97/00323 SEQ ID NO:1 or the anti-sense sequence or a biologically-effective fragment of either thereof.

A method of treatment of a patient in need of such treatment for a condition which is mediated by the biological activity of BS69 on a human BS69 binding substrate, comprising administration of an antibody against BS69 substantially as depicted in WO97/00323.

A compound that modulates the biological or pharmacological activity of BS69 on a human BS69 binding substrate identified by the method of the invention as described above.

A compound that modulates BS69 transcription or other BS69 activity, identified according to the methods of the invention as described above.

A pharmaceutical composition comprising a compound that modulates the biological 20 or pharmacological activity of BS69 on a human BS69 binding substrate identified by the method of the invention as described above.

A method of treatment of a patient in need of such treatment for a condition which is mediated by the pharmacological or biological activity of BS69 on a human BS69 binding substrate comprising administration of a modulating compound or pharmaceutical composition thereof identified by the method of the invention as described above.

Use of a polypeptide, nucleic acid, antibody or any other therapeutic agent substantially as depicted in WO97/00323, in the manufacture of a medicament for treating diseases mediated by TGF-β, particularly abnormal TGF-β expression.

The teaching in WO 97/00323 is incorporated herein by reference.

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### Example 1

# Identifying BS69 as a modulator of the TGF-β signalling pathway.

In order to identify novel modulators of the TGF-beta signalling pathway we

5 employed the two hybrid screening methodology. The two hybrid system can be used in order to detect expressed proteins from a cDNA library that interact with a protein of interest. The protein that we used as bait was Smad2. Smad2 is one of the pathway specific Smads and is known to lie on the TGF-beta pathway from receptor to nucleus. Smad2 has been shown to be in a complex with Smad4 and Fast-1 on the TGF-beta inducible Xenopus promoter, Mix.2.

### Two Hybrid Screen Construction

Human full length Smad2 was isolated by PCR from a human brain cDNA library (OriGene Technologies, Rockville MD.) and cloned into PCRScript® (Stratagene). SEQ ID Nos. 1 and 2 were the oligonucleotide primers used for the PCR synthesis.

The full length human Smad2 insert was excised from the PCRScript® using Sma I and Sal I restriction endonucleases and cloned into the two hybrid bait vector pGBD-1 (James, Genetics (1996) 144:1425-1436) resulting in a N-terminal Gal4 DNA binding domain fusion with full length human Smad2 (Gal4::Smad2). In order to verify that full length Smad2 was interaction competent, Xenopus Fast-1 and human Smad4 were cloned into the activation domain fusion vector pGAD-1 (AD::Fast1, AD::Smad4). It had been previously shown that these proteins will interact with human Smad2 (Chen, Nature (1997) 398(4):85-88). As such, the Smad2/Fast-1 and Smad2/Smad4 interaction was used as a positive control for the ability of Smad2 to interact with other proteins in the yeast two hybrid system.

Human full length Smad4 was isolated in a similar manner to Smad2 from a human skeletal muscle cDNA library using oligonucleotide primers corresponding to SEQ ID Nos. 3 and 4.

Xenopus Fast-1 was inserted into PCRScript® by amplification of a cloned Xenopus 30 Fast-1 sequence (C.Hill, ICRF, London) and then excised with Sma I and Bgl II restriction

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endonuclease and cloned into pGAD-1. The primers used for the PCR reaction were SEQ ID Nos. 5 and 6.

As a negative control human Smad1 was isolated and cloned into pGAD-1 (AD::Smad1). Smad1 does not interact with Smad2 *in vivo* or *in vitro* (Zhang et al. Nature 5 (1996) 383:168-172; Lagna, G., et al Nature (1996) 383:832-836). The primers used for the PCR reaction were SEQ ID Nos. 7 and 8.

The yeast two hybrid system used is that of Vidal et al., (PNAS USA (1996) 93:10321). The *S. cerevisiae* screening strain, MAV203, has three reporter genes (HIS3, URA3, and LacZ) stably integrated in single copy numbers at different loci in the yeast genome. Interaction of an activation domain fused protein with a DNA bound protein of interest will result in induction of the His3, Ura3, and LacZ reporter genes allowing growth of MAV203 on medium lacking histidine and uracil, and producing blue colonies when assayed with X-Gal (5-bromo-4-chloro-3-indolyl-b-D-galactopyranoside). MAV203 was transformed with combinations of the different control plasmids and were scored on their ability to grow on medium lacking uracil and histidine, and producing blue colour. This was done on a scale of +++, meaning very high, to -, meaning none.

Table 1 indicates that when GAL4::Smad2 is transformed into MAV203 on its own it cannot activate any of the reporter genes. If Smad2 is co-transformed with AD::Fast-1 a strong interaction is observed between the two as indicated by very high activation of the reporter genes. A similar strong interaction is observed between the Smad2 and AD::Smad4 fusion proteins in the two hybrid assay. Contrasting this, no interaction was observed between Smad2 and AD::Smad1 and none of the activation domain tagged proteins were able to activate the reporter genes on their own. This indicates that the GAL4::Smad2 fusion protein is functional in its ability to interact with other proteins.

A screen was then performed in order to identify proteins that interact with Smad2. MAV203 was co-transformed with GAL4::Smad2 and a human skeletal muscle two hybrid cDNA library (Clonetech). 5.9 x 10<sup>6</sup> independent co-transformants were assayed for their ability to interact with Smad2. Five different proteins were isolated and showed varying ability to interact with GAL4::Smad2 from very strong (+++, 1 isolate), strong (++, 2 isolates) and weak (+, 2 isolates) as assayed by their ability to activate all three reporter genes. The

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cDNA containing plasmids were isolated and co-transformed with GAL4::Smad2 as a check for "true positive". All five retained that ability to activate the three reporter genes.

The strongest interactor, Cl.51, was isolated independently in the screen 33 times. Sequence analysis of the longest isolate of Cl.51 indicated that it was a previously identified 5 protein BS69. BS69 was previously identified as a protein that interacts with the adenoviral 289R E1A gene product and in doing so inhibits its ability to activate transcription (Hateboer, R. et al., EMBO J. (1995) 14(13):3159-3169). BS69 is a 562 amino acid protein and has a single corresponding mRNA species of approximately 4.7 kb. The longest isolate of BS69 in the present two hybrid screen was 2200bp, encoding the C terminal region of the protein from 10 amino acid 450 to 562 and the 3' untranslated region. This area of the C terminus of BS69 contains the E1A interaction domain (Hateboer, R. et al., EMBO J. (1995) 14(13):3159-3169). It is therefore evident that the area of BS69 that mediates interaction with E1A also mediates interaction with Smad2.

In order to assess the specificity of the BS69/Smad2 interaction, the other members of the Smad family, as well as a totally unrelated protein, peroxisome proliferating antigen receptor gamma (PPAR-G), were examined for protein-protein interaction with BS69 in the two hybrid system. Each of the proteins were cloned from PCR reactions in a manner similar to that for Smad2 into the pGBD-1 vector in order to make GAL4 DNA binding domain fusions. Smad3 was cloned using PCR primers corresponding to SEQ ID Nos. 9 and 10.

20 Smad5 was cloned using PCR primers corresponding to SEQ ID Nos. 11 and 12. Smad6 was cloned using PCR primers corresponding to SEQ ID Nos. 13 and 14. Smad 7 was cloned using PCR primers corresponding to SEQ ID Nos. 15 and 16.

Table 2 summarises the results from the BS69 specificity two hybrid analysis. BS69 interacts very strongly with Smad2 and Smad3. A very weak (+/-) interaction is obtained with Smad1 which may not be physiologically relevant. No interaction is observed with Smad4, Smad5, Smad6, or Smad7. BS69 does not interact with PPAR-G and cannot activate the reporter genes on its own. This indicates that the BS69 interaction observed is specific to Smad2 and Smad3 (possibly Smad1) suggesting a role for BS69 in modulating the activity of TGF-beta through Smad2 and/or Smad3. As BS69 was identified as a protein that interacts with E1A, inhibiting its ability to activate transcription, the same may be true in relation to the TGF-beta pathway. Smad2 and Smad3 have both been shown to contain transcription

activation function. They heterodimerise with Smad4, translocate to the nucleus, and activate TGF-beta responsive genes. BS69 may function cellularly as an inhibitor of TGF-beta induced transcriptional activation by interacting with Smad2/Smad3 and inhibiting their ability to activate transcription.

It will be apparent to the person skilled in the art that use of the specific vectors and strains as described in this example is not essential. Other commercially available yeast two hybrid systems, using slightly different vectors and host strains, could be used. For example, Mav 203 yeast strain could be replaced by Clontech CG-1945 yeast strain and the pGAD and pGBD vectors could be replaced by the Clontech pGAD424 and pGBT9 vectors respectively.

Table 1: GAL4::Smad2 Interaction Verification

Co-Transformant Strength

-	-
AD::Fast-1	+++
AD::Smad4	+++
AD::Smad1	-

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15 MAV203 were transformed with GAL4::Smad2 and the indicated co-transformant. They were then assayed for strength of interaction on their ability to activate the URA3, HIS3, and LacZ reporter gene.

**Table 2: BS69 Specificity Interaction** 

Co-Transformant Strength

+/-
+++
+++
-
-

- 16 -

GAL4::Smad6	-
GAL4::Smad7	
GAL4::PPAR-G	-

MAV203 were transformed with AD::BS69 and the indicated co-transformant and assayed for ability to interact. Strength of interaction is assayed by ability to activate URA3, HIS3, and LacZ reporter gene.

5

### Example 2.

## Confirming that BS69 interacts with Smad2 and Smad3 in vivo.

## Co-immunoprecipitation and Western blot analysis.

- The inventors have used pGen-Ires-neo as their chosen mammalian expression vector. The salient features of this construct are, in sequence: the backbone vector of plasmid pCI (Promega) with a CMV promoter for high level expression, a synthetic splice donor/acceptor sequence to ensure correct processing of transcripts, an engineered polylinker site to facilitate cloning of the gene to be expressed and the IRES element from encephalomyocarditis virus (gift from Ira Pastan; see Sugimoto et al., Biotechnology (1994) 12:694) fused in frame to the initiating ATG of the neo gene from pcDNA3 (Invitrogen). It will be apparent to the person skilled in the art of mammalian expression that the use of this specific vector is not essential to the working of this example or the invention. Various alternative vectors, such as pIRES-neo (Clontech) and pCL-neo (Promega) could also be used.
- PCR was used to create N-terminal Flag-tagged full length BS69 and truncated BS69 (amino acids 443 562), to be subcloned into pGen-Ires-neo expression vector. Full length hSmad-2, hSmad-3 without a tag, prepared according to Example 1, were also subcloned into pGen-Ires-neo.
- The Flag octapeptide (5'-Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys-3'; IBI Flag Biosystem; SEQ ID No. 17) has a distinct anti Flag M1 monoclonal antibody binding site and has been carefully designed for easy access on the surface of the expression protein, allowing easy detection and purification.

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### PCR cloning strategy

		primer
Delta BS69	5'-EcoRI-Nhel-ATG-aa.412	SEQ ID No. 18
Delta BS69 - Pag tagged	5'-EcoRI-NheI-ATG- Flag epitope-	SEQ ID No. 19
Full length BS69	5'-EcoRI-NheI-ATG-aa.2	SEQ ID No. 20
Full length BS69 - Flag	5'-EcoRI-NheI-ATG- Flag epitope-	SEQ ID No. 21
tagged	aa.2	
BS69 3'end	3'-ClaI-XbaI-Stop codon	SEQ ID No. 22

BS69 untagged was cloned by RT-PCR from a human placenta cDNA library. Flag tagged BS69 was made by PCR from the full length untagged BS69. Truncated BS69 tagged and untagged constructs were made by PCR using the Yeast-two-hybrid cDNA clone identified above.

Cos-1 cells were transfected with respective DNAs using Lipofectamine Plus Reagent (Life Technologies). 48 hours after transfection, cells were treated with 15ng/ml of TGFbeta1 (R&D Systems) for 1 hour, and then lysed in 4 mls of ice cold RIPA buffer (150mls 10 PBS, 1.5ml Triton-X-100, 0.75g sodium deoxycholate, 0.75ml 20% SDS). Cell lysates were collected by centrifugation and precleared with 1µg normal mouse IgG (Santa Cruz) and 20µl Protein A/G sepharose (Sigma). Co-immunoprecipitation was performed on 1 ml of lysate incubated at 4°C with 20µl of alpha-Smad2/3 polyclonal antibody (Santa Cruz) for 4 hours followed by the addition of 40µl Protein A/G Sepharose (Sigma). After overnight incubation 15 at 4°C, the beads were spun down and washed 4 times with lysis buffer, then resuspended in 50µl Laemmli sample buffer (Sigma) and boiled for 5 minutes. Half of the sample was separated by polyacrylamide gel electrophoresis 4-15% Tris-HCl (Biorad) and transferred overnight to nitrocellulose membrane (Amersham). The filter was blocked in TBS buffer (20mM Tris base, 137mM NaCl, 3.8mM HCl, pH 7.6) with 0.1% Tween-20 and 5% non-fat 20 powdered milk for 2 hours at room temperature, incubated with the primary antibody alpha-Flag monoclonal antibody (Santa Cruz) in a dilution of 1:2000 in TBS-Tween. The second antibody conjugated to HRP in a 1:1000 dilution for 1 hour. The filter was washed in TBS-Tween and detection was performed using enhanced chemiluminescence (Amersham).

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It was found that both full length BS69 and truncated BS69 bind to hSmad-2, and to hSmad-3. This experiment therefore confirms that the BS69 interaction with hSmad-2/-3 identified in the yeast two-hybrid screening is a true *in vivo* interaction.

### 5 Example 3

### Mammalian reporter assay.

pGen-Ires-neo vector was used for expression of untagged full length hSmad-2, hSmad-3, hSmad-4, BS69 and untagged truncated BS69.

The 959bp fragment corresponding to the promoter region (-811bp to +148bp) of the PAI-1 gene was PCRed from human genomic template DNA using SEQ ID Nos 23 and 24 primers. These primers allow addition of HindIII restriction sites at the 5'and the 3'ends to facilitate subcloning into the pGL3 basic vector (Promega - Luciferase reporter vectors - Technical manual, part#TM033).

The p300/CBP co-activator expression construct comprises the 1-7326bp nucleotide sequence of murine CREB Binding Protein, as published in Genbank (g435854), obtained by PCR from mouse brain mRNA using primers corresponding to SEQ ID Nos. 25 and 26. The 7,326kb BamHI-NotI fragment was subcloned into pRc/RSV expression plasmid (Invitrogen).

On day one, 10<sup>6</sup> HepG2 cells (human hepatocellular carcinoma cells - Origin ECACC 85011430) were seeded per well of a 6 well plate in DMEM medium, 10%FCS, Glutamine and Penicillin/Streptavidin. Cells were transfected on day two by adding 4.75ug DNA/ well and 4ul of lipofectamine/ 4ul Reagent Plus (Life Technologies) per well as recommended by the manufacturer. Serum was added to the cells after 4 hours from. The medium was replaced with serum free medium the next day, and stimulated 7 hours later with 7.5ng/ml of TGF-

25 beta1 overnight. Cytosol extract and dual luciferase assay were done the following day. The dual luciferase reporter assay system (Promega, technical manual part#TM040) was used as recommended by the manufacturer, and using 5ng of pBRL per well for normalisation of the assay.

The activity of the luciferase reporter gene relates to the activity of the PAI-1 promoter activation. PAI activity is minimal in the absence of TGF-β stimulation, but increases upon TGF-β stimulation. No effect of the above DNA constructs was observed in the absence of

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TGF-β stimulation, therefore all the following experiments were done with TGF-β stimulation. When hSmad-2 or hSmad-3 were transfected alone into HepG2 cells, an additional increase in promoter activation was observed. There was no change when hSmad-4, p300/CBP, hSmad4+p300/CBP, fl BS69 or d-BS69 were transfected alone.

hSmad-4 is known to form a complex with activated Smad-2/or-3, and therefore additional hSmad-4 (limiting factor) together with hSmad-2/or-3 will increase PAI activity, as found in this assay and observed previously.

5

P300/CBPhas been shown to be a transciptional co-activator implicated in TGF-β signalling promoter activation, and when added to Smad-2/or-3 together with Smad-4 induces an additional increase in PAI activation (about 15%), as seen in our assay and observed previously.

Full length BS69 induces significant additional increase in PAI activation (up to 60%), when added to HepG2 cells together with hSmad-2/or-3, or hSmad-2/or-3 + hSmad-4 + p300/CBP.

Truncated BS69 acts like a dominant-negative form of BS69 when added to HepG2 cells together with hSmad-2/or-3, or hSmad-2/or-3 + hSmad-4 + p300/CBP. A small reduction of PAI activity is observed which probably correspond to the inhibitory effect of truncated BS69 to the full length constitutive basal expression of BS69 in HepG2 cells.

We have found using co-immunoprecipitation studies that both full length and truncated BS69 can bind Smad-2/or-3, and that full length BS69 is able to induce additional PAI promoter activation when tested in a mammalian reporter assay.

#### **Claims**

5

10

- 1. A method for identifying modulators of BS69 activity, which method comprises contacting an assay system, capable of presenting information on the effects of a test compound on the activity of BS69 or a derivative thereof, with a test compound and measuring the activity of BS69.
- 2. A method as claimed in claim 1, wherein BS69 activity refers to the ability of BS69 or a fragment thereof to bind to a BS69 binding protein.
- 3. A method as claimed in claim 2 wherein the BS69 binding protein is one selected from the group consisting of: Smad 2, Smad 3, a complex of Smad 2 and Smad 4, a complex of Smad 3 and Smad 4, or fragments thereof, or PAI-1 promoter element.
- 15 4. A cell or cell line comprising a reporter gene under the control of a BS69 transcription factor dependent promoter.
- A method for identifying modulators of BS69 transcription which method comprises contacting a cell or cell line as claimed in claim 4 with a test compound, said cell or cell line supplied with exogenous or endogenous BS69, and determining the effect on BS69 transcription by the test compound by reference to enhanced or reduced expression of the reporter gene.
- A method of treatment of a patient in need of such treatment for a condition which is
   mediated by the biological or pharmacological activity of BS69 on a human BS69 binding substrate, comprising administration of a polypeptide substantially as depicted in WO97/00323 SEQ ID NO:2 or a pharmacologically active fragment thereof.
- A method of treatment of a patient in need of such treatment for a condition which is
   mediated by the biological activity of BS69 on a human BS69 binding substrate,
   comprising administration of a nucleic acid substantially as depicted in WO97/00323

SEQ ID NO:1 or the anti-sense sequence or a biologically-effective fragment of either thereof.

8. A compound that modulates BS69 transcription or other BS69 activity identified
5 according to the method as described in any of claims 1, 2, 3 and 5.

10

 A pharmaceutical composition comprising a compound that modulates the biological or pharmacological activity of BS69 on a human BS69 binding substrate identified according to the method as described in any of claims 1, 2, 3 and 5.

10. A method of treatment of a patient in need of such treatment for a condition which is mediated by the pharmacological or biological activity of BS69 on a human BS69 binding substrate comprising administration of a modulating compound or pharmaceutical composition as claimed in claims 8 or 9.

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-7-

```
<220>
    <223> Description of Artificial Sequence: Synthetic
          single-stranded oligonucleotide primer sequence
 5 <400> 18
    gtgaattcgc tagcatgcat cggagcaccc agaccacaaa
                                                                       40
    <210> 19
10 <211> 62
    <212> DNA
    <213> Artificial Sequence
    <220>
15 <223> Description of Artificial Sequence: Synthetic
          single-stranded oligonucleotide primer sequence
    <400> 19
    tcgaattcgc tagcatggac tacaaggacg acgatgacaa gcatcggagc acccagacca 60
20 ca
                                                                       62
    <210> 20
    <211> 37
25 <212> DNA
    <213> Artificial Sequence
    <220>
    <223> Description of Artificial Sequence: Synthetic
30
          single-stranded oligonucleotide primer sequence
    <400> 20
   gtgaattcgc tagcatgtct cgagtccacg gtatgca
                                                                       37
35
   <210> 21
   <211> 62
   <212> DNA
   <213> Artificial Sequence
40
   <220>
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<223> Description of Artificial Sequence: Synthetic
           single-stranded oligonucleotide primer sequence
    <400> 21
 5 togaattogo tagoatgaca tacaaggacg acgatgacaa gtotogagto cacggtatgo _{60}
    <210> 22
10 <211> 38
    <212> DNA
    <213> Artificial Sequence
    <220>
15 <223> Description of Artificial Sequence: Synthetic
          single-stranded oligonucleotide primer sequence
    <400> 22
    gtatcgattc tagatcatct tttccggcgg caggtgcg
                                                                        38
20
    <210> 23
    <211> 25
    <212> DNA
25 <213> Artificial Sequence
    <220>
    <223> Description of Artificial Sequence: Synthetic
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30
    <400> 23
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                                                                       25
35 <210> 24
    <211> 28
   <212> DNA
    <213> Artificial Sequence
40 <220>
   <223> Description of Artificial Sequence: Synthetic
```

WO 00/28323 PCT/GB99/03648

38

-9-

single-stranded oligonucleotide primer sequence

```
<400> 24
    ggaaaagctt tctcctacct gaagttct
                                                                       28
 5
    <210> 25
    <211> 31
    <212> DNA
10 <213> Artificial Sequence
    <220>
    <223> Description of Artificial Sequence: Synthetic
          single-stranded oligonucleotide primer sequence
15
    <400> 25
    ggatccagaa tggccgagaa cttgctggac g
                                                                      31
20 <210> 26
    <211> 38
   <212> DNA
   <213> Artificial Sequence
25 <220>
```

<223> Description of Artificial Sequence: Synthetic single-stranded oligonucleotide primer sequence

<400> 26

30 gcggccgcta caaaccctcc acaaactttt ctagtgtg

ional Application No.

PCT/GB 99/03648 A. CLASSIFICATION OF SUBJECT MATTER IPC 7 G01N33/50 C12N C12N5/10 C1201/68 According to International Patent Classification (IPC) or to both national classification and IPC **B. FIELDS SEARCHED** Minimum documentation searched (classification system followed by classification symbols) IPC 7 G01N C12Q Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practical, search terms used) C. DOCUMENTS CONSIDERED TO BE RELEVANT Category \* Citation of document, with indication, where appropriate, of the relevant passages Relevant to claim No. χ KUROZUMI K ET AL: "BRAM1, a BMP 1-3,8,9receptor-associated molecule involved in BMP signalling." GENES TO CELLS, (1998 APR) 3 (4) 257-64. XP000870308 cited in the application page 259, paragraph 3 the whole document 4-7,10WO 97 00323 A (PROLIFIX LTD ; VER NL KANKER X 1,2,8,9 INST (NL); HATEBOER GUUS (NL); BERNARD) 3 January 1997 (1997-01-03) cited in the application page 13, line 10 - line 12

Patent family members are listed in annex.
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention  "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone  "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.  "&" document member of the same patent family
Date of mailing of the international search report
04/02/2000
Authorized officer  Gundlach, B

2





Inte .cional Application No PCT/GB 99/03648

C.(Continu	ation) DOCUMENTS CONSIDERED TO BE RELEVANT	101/06 99/03048
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HATEBOER G ET AL: "BS69, a novel adenovirus E1A-associated protein that inhibits E1A transactivation." EMBO JOURNAL, (1995 JUL 3) 14 (13) 3159-69. , XP000579801 cited in the application the whole document	1,2,8,9
X	KEETON ET AL: "Identification of regulatory sequences in the type 1 plasminogen activator inhibitor gene responsive to transforming growth factor beta"  JOURNAL OF BIOLOGICAL CHEMISTRY, US, AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, vol. 266, no. 34, page 23048-23052-23052 XP002110964  ISSN: 0021-9258 page 23048, column 2, paragraph 5 -page 23049, column 1, paragraph 2	4
X	DENNLER S ET AL: "Direct binding of Smad3 and Smad4 to critical TGF beta-inducible elements in the promoter of human plasminogen activator inhibitor-type 1 gene" EMBO JOURNAL,GB,OXFORD UNIVERSITY PRESS, SURREY, vol. 17, no. 11, page 3091-3100 XP002110965 ISSN: 0261-4189 page 3092, paragraph 2	4
X	DE CAESTECKER M P ET AL: "Characterization of functional domains within Smad4/DPC4" JOURNAL OF BIOLOGICAL CHEMISTRY,US,AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, vol. 272, no. 21, page 13690-13696 XP002084021 ISSN: 0021-9258 page 13692, column 2, line 8 -page 13693, column 1, line 6; figure 2B	4
	YINGLING ET AL: "Tumor suppressor Smad4 is a transforming growth factor b-inducible DNA binding protein" MOLECULAR AND CELLULAR BIOLOGY, US, WASHINGTON, DC, vol. 17, no. 12, page 7019-7028-7028 XP002106769 ISSN: 0270-7306 the whole document	4





Inte. .onal Application No PCT/GB 99/03648

ategory ·	ation) DOCUMENTS CONSIDERED TO BE RELEVANT  Citation of document, with indication, where appropriate, of the relevant passages	In .
,	те relevant passages	Relevant to claim No.
, X	WO 99 40220 A (GAUTHIER JEAN MICHEL ;GLAXO GROUP LTD (GB)) 12 August 1999 (1999-08-12) page 29	4
	·	
	,	·
	•	



international application No.

PCT/GB 99/03648

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)
This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:
1. X Claims Nos.:  because they relate to subject matter not required to be searched by this Authority, namely:  Although claims 6 and 7 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition. Rule 39.1(iv) PCT - Method for treatment of the human or animal body by therapy  2. Claims Nos.:  because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
Claims Nos.:     because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).
Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)
This International Searching Authority found multiple inventions in this international application, as follows:
As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
Remark on Protest  The additional search fees were accompanied by the applicant's protest.  No protest accompanied the payment of additional search fees.

International Application No. PCT/GB 99 \03648

## FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

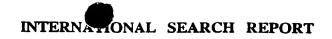
Continuation of Box I.1

Although claims 6 and 7 are directed to a method of treatment of the human body, the search has been carried out and based on the alleged effects of the used compounds.

\_\_\_\_\_

Continuation of Box I.1

Rule 39.1(iv) PCT - Method for treatment of the human or animal body by therapy



Information on patent family members

0

Inte ional Application No PCT/GB 99/03648

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9700323	A	03-01-1997	AU 6230496 A EP 0833924 A US 5985283 A	15-01-1997 08-04-1998 16-11-1999
WO 9940220	A	12-08-1999	NONE	



## **PCT**

## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's o	or ager	nt's file reference	EOD EUDTHED ACT		Notification of Transmittal of International	
PHM 70421/WO		FOR FURTHER ACT	Preli	minary Examination Report (Form PCT/IPEA/416)		
International application No. International fili				ny/month/year)	Priority date (day/month/year)	
PCT/GB9	9/036	648	04/11/1999		10/11/1998	
International G01N33/5		nt Classification (IPC) or n	ational classification and IPC			
Applicant						
ASTRAZE	ENEC	CA AB et al.				
			nination report has been p according to Article 36.	repared by th	is International Preliminary Examining Authority	
2. This R	EPO	RT consists of a total o	f 8 sheets, including this	cover sheet.		
be (s	<ul> <li>This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).</li> <li>These annexes consist of a total of sheets.</li> </ul>					
3. This re		contains indications rel	lating to the following item	s:		
11		Priority				
111	$\boxtimes$		· -	elty, inventive	e step and industrial applicability	
IV	_	Lack of unity of invent				
\ \ \	×		under Article 35(2) with regions suporting such stater		y, inventive step or industrial applicability;	
VI		Certain documents ci				
VII			international application			
VIII	Ø	Certain observations	on the international applica	ation		
Date of sub	missio	on of the demand		Date of comple	etion of this report	
16/05/20	00			15.11.2000		
	exami	g address of the internation ning authority:	nal	Authorized offi	CET JUNEAU COLS MICHAEL E	
0))	D-80	pean Patent Office 0298 Munich		Moreno de	Vega, C	
		+49 89 2399 - 0 Tx: 5236 +49 89 2399 - 4465	56 epmu d		10 20 2200 7400	

Telephone No. +49 89 2399 7486

Fax: +49 89 2399 - 4465

International application No. PCT/GB99/03648

## I. Basis of the report

1.	resp the	oonse to an invitation	awn on the basis of (substitute sheets which have been furnished to the receiving Office in n under Article 14 are referred to in this report as "originally filed" and are not annexed to not contain amendments (Rules 70.16 and 70.17).):				
	1-19	9	as originally filed				
	Clai	ims, No.:					
	1-10	)	as originally filed				
2.			uage, all the elements marked above were available or fumished to this Authority in the nternational application was filed, unless otherwise indicated under this item.				
	The	se elements were a	vailable or fumished to this Authority in the following language: , which is:				
		• • •	ranslation furnished for the purposes of the international search (under Rule 23.1(b)).				
		the language of pu	blication of the international application (under Rule 48.3(b)).				
		the language of a t 55.2 and/or 55.3).	ranslation furnished for the purposes of international preliminary examination (under Rule				
3.			leotide and/or amino acid sequence disclosed in the international application, the yexamination was carried out on the basis of the sequence listing:				
		contained in the in	ternational application in written form.				
		filed together with	the international application in computer readable form.				
		fumished subsequ	ently to this Authority in written form.				
		furnished subsequ	ently to this Authority in computer readable form.				
		The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.					
		The statement that listing has been fu	t the information recorded in computer readable form is identical to the written sequence rnished.				
4.	The	amendments have	resulted in the cancellation of:				
		the description,	pages:				
		the claims,	Nos.:				
		the drawings,	sheets:				
5.			en established as if (some of) the amendments had not been made, since they have been beyond the disclosure as filed (Rule 70.2(c)):				

International application No. PCT/GB99/03648

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6.	Ado	Additional observations, if necessary:				
Ш.	Noi	n-establishment of opin	ion witl	n regard	to novelty, inventive step and industrial applicability	
	-	lestions whether the clain e industrially applicable h			pears to be novel, to involve an inventive step (to be non-obvious), camined in respect of:	
		the entire international a	pplication	on.		
	×	claims Nos. 6, 7, 10.				
be	caus	se:				
	×				said claims Nos. 6, 7, 19 relate to the following subject matter which nary examination ( <i>specify</i> ):	
		the description, claims of that no meaningful opinion		• .	icate particular elements below) or said claims Nos. are so unclear ned (specify):	
		the claims, or said claim could be formed.	ıs Nos.	are so in	nadequately supported by the description that no meaningful opinion	
		no international search	report h	as been	established for the said claims Nos	
2.	and	-		-	ination report cannot be carried out due to the failure of the nucleotide y with the standard provided for in Annex C of the Administrative	
		the written form has not	been fu	ımished o	or does not comply with the standard.	
		the computer readable t	orm has	s not bee	en furnished or does not comply with the standard.	
V.		asoned statement unde ations and explanations			vith regard to novelty, inventive step or industrial applicability; ch statement	
1.	Sta	tement				
	Nov	velty (N)	Yes: No:		3, 5, 6 1, 2, 4, 7-10	
	Inv	entive step (IS)	Yes: No:	Claims Claims		
	Ind	ustrial applicability (IA)	Yes:	Claims	1-5, 8, 9	

International application No. PCT/GB99/03648

No: Claims

2. Citations and explanations see separate sheet

#### VI. Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

### VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted: see separate sheet

#### VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made: see separate sheet

#### Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Claims 6, 7 and 10 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subjectmatter of these claims (Article 34(4)(a)(i) PCT).

#### Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

- D1: KUROZUMI K ET AL: 'BRAM1, a BMP receptor-associated molecule involved in BMP signalling.' GENES TO CELLS, (1998 APR) 3 (4) 257-64., cited in the application
- D2: WO 97 00323 A (PROLIFIX LTD ; VER NL KANKER INST (NL); HATEBOER GUUS (NL); BERNARD) 3 January 1997 (1997-01-03) cited in the application
- D3: WO 99 40220 A (GAUTHIER JEAN MICHEL ;GLAXO GROUP LTD (GB)) 12 August 1999 (1999-08-12)
- D4: HATEBOER G ET AL: 'BS69, a novel adenovirus E1A-associated protein that inhibits E1A transactivation.' EMBO JOURNAL, (1995 JUL 3) 14 (13) 3159-69, cited in the application
- D5: KEETON ET AL: 'Identification of regulatory sequences in the type 1 plasminogen activator inhibitor gene responsive to transforming growth factor beta' JOURNAL OF BIOLOGICAL CHEMISTRY, US, AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, vol. 266, no. 34, page 23048-23052-23052 ISSN: 0021-9258
- D6: DENNLER S ET AL: 'Direct binding of Smad3 and Smad4 to critical TGF beta-inducible elements in the promoter of human plasminogen activator

inhibitor-type 1 gene' EMBO JOURNAL,GB,OXFORD UNIVERSITY PRESS, SURREY, vol. 17, no. 11, page 3091-3100 ISSN: 0261-4189

D7: DE CAESTECKER M P ET AL: 'Characterization of functional domains within Smad4/DPC4' JOURNAL OF BIOLOGICAL CHEMISTRY,US,AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, vol. 272, no. 21, page 13690-13696 ISSN: 0021-9258

## 1. Novelty (Article 33(2) PCT)

D1 discloses the isolation of a cytoplasmic molecule associated with the bone morphogenetic protein (BMP) type A receptor (BMPR-IA), this molecule being designated as BMP receptor associated molecule (BRAM1). BRAM1 is an alternatively spliced form of BS69 (see Abstract). The interaction of BRAM1 with other molecules involved in TAK1 (TGF-β activated kinase 1) mediated BMP signalling cascade by the use of the yeast two-hybrid system is examined (see page 261-page 262, 1st paragraph, page 262, right column, 3rd paragraph). This document appears to be novelty destroying for claims 1, 2 and 8.

D2 discloses assaying for promoters or inhibitors of dimerisation of BS69 with a complexor, e. g. a protein (see page 13, lines 10-12, page 21 lines 10-24, claims 24-29), and candidate therapeutic agents and methods of gene therapy using a polynucleotide including the SEQ ID NO: 1 (page 12, claims 31 and 32). This document appears to be novelty destroying for claims 1, 2, 7-10

D3 describes the cloning of BS69, which coimmunoprecipitates with E1A protein in adenovirus transformed 293 cells (see abstract, page 3160 right column). This document appears to be novelty destroying for claims 1, 2 and 8.

D4 (see page 23048, right column, 5th paragraph - page 23049, left column, 2nd paragraph), D5 (Abstract, page 3092, left column 2nd paragraph), D6 (Figure 2) and D7 (see Materials and Methods) disclose a cell line comprising a reporter gene under the control of a BS69 transcription factor

dependent promoter. These documents appear to be novelty destroying for claim 4.

Thus, claims 1, 2, 4, 7-10 do not meet the requirements of Article 33(2) PCT.

2. Inventive step (Article 33(3) PCT)

Claim 6 is not considered to be inventive on the light of D1, which discloses the SEQ ID NO: 2 and a similar method using another sequence.

A cell line comprising a reporter gene under the control of a BS69 transcription factor dependent promoter is already described in D4-D7. Thus, claim 5 is obvious on the light of D4-D7 and is considered not to be inventive.

Claim 3 is considered to be inventive. D2, considered to be the most relevant prior art, fail to describe the relationship between BS69 and Smad proteins. The technical problem to be solved by claim 3 is the provision of methods for identifying modulators of BS69 activity. The solution provided by claim 3 is based on the discovery that the protein BS69 complexes with the Smad 2 and 3 proteins. This solution was neither disclosed nor suggested in the prior art.

3. For the assessment of the present claims 6, 7 and 10 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

## INTERNATIONAL PRELIMINARY

International application No. PCT/GB99/03648

### **EXAMINATION REPORT - SEPARATE SHEET**

#### Re Item VI

#### Certain documents cited

Certain published documents (Rule 70.10)

Application No Patent No

Publication date (day/month/year)

Filing date (day/month/year) Priority date (valid claim) (day/month/year)

WO 99/40220

12.08.1999

04.02.1999

06.02.1998

The intermediate document referred above (= D8) disclose a cell line transformed with a fragment of the PAI-1 promoter. This document, therefore, would appear to disclose or make obvious the subject matter of claim 4.

However, it is considered that the priority of the present application is validly claimed. Should the present application be entered into the regional phase, the above document could be relevant to the question of novelty.

#### Re Item VII

## Certain defects in the international application

Reference to prior art being incorporated by reference is not allowed, as the application should be self-contained. Therefore, sentences like "...is incorporated herein by reference" in page 11 line 29 contravene Guidelines C II, 4.17 PCT.

#### Re Item VIII

#### Certain observations on the international application

- Claim 1 is not in line with the description (Article 6 PCT), which on page 9, 1. lines 26-31, discloses "a fragment of BS69", but disclaims BRAM1, which can be regarded as a fragment or derivative of BS69.
- 2. Claims 6 and 7 are not clear: it is not possible to make reference in the claims to SEQ ID disclosed in other patent documents, as the application should be self-contained. Therefore, these claims do not meet the requirements of Article 6 PCT.
- Claim 1 contains no technical feature and tries to define the subject-matter in 3. vague and imprecise terms (Article 6 PCT).



## **PCT**

REC'D 17 NOV 2000

**WIPO** PCT

See Notification of Transmittal of International

## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

PHM 70421/WO FOR			FOR FURTHER ACT	ION Preliminar	y Examination Report (Form PCT/IPEA/416)		
International application No. International filing date			International filing date (da	y/month/year)	Priority date (day/month/year)		
PCT/GB99			04/11/1999		10/11/1998		
International Patent Classification (IPC) or national classification and IPC G01N33/50							
Applicant	Applicant						
ASTRAZE	ASTRAZENECA AB et al.						
1. This int	This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.						
2. This R	EPO	RT consists of a total of	8 sheets, including this	cover sheet.			
be (so	<ul> <li>□ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).</li> <li>These annexes consist of a total of sheets.</li> </ul>						
	_		ating to the following item				
I ⊠ Basis of the report II □ Priority							
11 111			opinion with regard to nov	velty, inventive ste	p and industrial applicability		
iv		Lack of unity of invent		•			
v	Ø	Reasoned statement u	under Article 35(2) with re ions suporting such state	gard to novelty, in ment	ventive step or industrial applicability;		
VI	$\boxtimes$	Certain documents ci	ted				
VII	$\boxtimes$		international application				
VIII	×	Certain observations	on the international applic	ation			
i							
Date of sub	missio	on of the demand		Date of completion	of this report		
16/05/20	00		:	15.11.2000			
	exam	g address of the internation ining authority:	nal	Authorized officer	St. M. S.		
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d				Moreno de Veç	The state of the s		
Fax: +49 89 2399 - 4465				Telephone No. +49	89 2399 /486		

Applicant's or agent's file reference

International application No. PCT/GB99/03648

		s of the report
1.	resp the r	report has been drawn on the basis of (substitute sheets which have been furnished to the receiving Office in onse to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to report since they do not contain amendments (Rules 70.16 and 70.17).): cription, pages:
	1-19	as originally filed
	Clai	ms, No.:
	1-10	as originally filed
2.	With	n regard to the <b>language</b> , all the elements marked above were available or fumished to this Authority in the juage in which the international application was filed, unless otherwise indicated under this item.
	The	se elements were available or furnished to this Authority in the following language: , which is:
		the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
		the language of publication of the international application (under Rule 48.3(b)).
		the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).
3.	Wit inte	h regard to any <b>nucleotide and/or amino acid sequence</b> disclosed in the international application, the mational preliminary examination was carried out on the basis of the sequence listing:
		contained in the international application in written form.
		filed together with the international application in computer readable form.
		furnished subsequently to this Authority in written form.
		furnished subsequently to this Authority in computer readable form.
		The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
		The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

5. This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

☐ the description,

☐ the claims,☐ the drawings,

4. The amendments have resulted in the cancellation of:

pages:

Nos.:

sheets:

International application No. PCT/GB99/03648

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

		report.)			
6.	Add	itional observations, if nec	essary:		
					to novelty, inventive step and industrial applicability
Th or	e qu to be	estions whether the claime industrially applicable ha	ed inver ive not b	ntion app been exai	pears to be novel, to involve an inventive step (to be non-obvious), amined in respect of:
		the entire international ap	plicatio	n.	
	×	claims Nos. 6, 7, 10.			
be	caus	se:			
	Ø	the said international app does not require an inter see separate sheet	olication national	, or the sa	said claims Nos. 6, 7, 19 relate to the following subject matter which nary examination ( <i>specify</i> ):
		the description, claims or drawings (indicate particular elements below) or said claims Nos. are so unclear that no meaningful opinion could be formed (specify):			
		the claims, or said claims could be formed.	s Nos.	are so ina	nadequately supported by the description that no meaningful opinion
		no international search r	eport ha	as been e	established for the said claims Nos
2.	A meaningful international preliminary examination report cannot be carried out due to the failure of the nucleotic and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:				
		the written form has not	been fu	mished o	or does not comply with the standard.
					en furnished or does not comply with the standard.
V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement					
1	. Sta	atement			
	No	ovelty (N)	Yes: No:		3, 5, 6 1, 2, 4, 7-10
	Inv	ventive step (IS)	Yes: No:	Claims Claims	
	Ind	dustrial applicability (IA)	Yes:	Claims	1-5, 8, 9

International application No. PCT/GB99/03648

No: Claims

2. Citations and explanations see separate sheet

#### VI. Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

## VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted: see separate sheet

## VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made: see separate sheet

### Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Claims 6, 7 and 10 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subjectmatter of these claims (Article 34(4)(a)(i) PCT).

### Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

- D1: KUROZUMI K ET AL: 'BRAM1, a BMP receptor-associated molecule involved in BMP signalling.' GENES TO CELLS, (1998 APR) 3 (4) 257-64., cited in the application
- D2: WO 97 00323 A (PROLIFIX LTD ; VER NL KANKER INST (NL); HATEBOER GUUS (NL); BERNARD) 3 January 1997 (1997-01-03) cited in the application
- D3: WO 99 40220 A (GAUTHIER JEAN MICHEL ;GLAXO GROUP LTD (GB)) 12 August 1999 (1999-08-12)
- D4: HATEBOER G ET AL: 'BS69, a novel adenovirus E1A-associated protein that inhibits E1A transactivation.' EMBO JOURNAL, (1995 JUL 3) 14 (13) 3159-69, cited in the application
- D5: KEETON ET AL: 'Identification of regulatory sequences in the type 1 plasminogen activator inhibitor gene responsive to transforming growth factor beta' JOURNAL OF BIOLOGICAL CHEMISTRY, US, AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, vol. 266, no. 34, page 23048-23052-23052 ISSN: 0021-9258
- D6: DENNLER S ET AL: 'Direct binding of Smad3 and Smad4 to critical TGF beta-inducible elements in the promoter of human plasminogen activator

inhibitor-type 1 gene' EMBO JOURNAL,GB,OXFORD UNIVERSITY PRESS, SURREY, vol. 17, no. 11, page 3091-3100 ISSN: 0261-4189

D7: DE CAESTECKER M P ET AL: 'Characterization of functional domains within Smad4/DPC4' JOURNAL OF BIOLOGICAL CHEMISTRY,US,AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, vol. 272, no. 21, page 13690-13696 ISSN: 0021-9258

## 1. Novelty (Article 33(2) PCT)

D1 discloses the isolation of a cytoplasmic molecule associated with the bone morphogenetic protein (BMP) type A receptor (BMPR-IA), this molecule being designated as BMP receptor associated molecule (BRAM1). BRAM1 is an alternatively spliced form of BS69 (see Abstract). The interaction of BRAM1 with other molecules involved in TAK1 (TGF-β activated kinase 1) mediated BMP signalling cascade by the use of the yeast two-hybrid system is examined (see page 261-page 262, 1st paragraph, page 262, right column, 3rd paragraph). This document appears to be novelty destroying for claims 1, 2 and 8.

D2 discloses assaying for promoters or inhibitors of dimerisation of BS69 with a complexor, e. g. a protein (see page 13, lines 10-12, page 21 lines 10-24, claims 24-29), and candidate therapeutic agents and methods of gene therapy using a polynucleotide including the SEQ ID NO: 1 (page 12, claims 31 and 32). This document appears to be novelty destroying for claims 1, 2, 7-10

D3 describes the cloning of BS69, which coimmunoprecipitates with E1A protein in adenovirus transformed 293 cells (see abstract, page 3160 right column). This document appears to be novelty destroying for claims 1, 2 and 8.

D4 (see page 23048, right column, 5th paragraph - page 23049, left column, 2nd paragraph), D5 (Abstract, page 3092, left column 2nd paragraph), D6 (Figure 2) and D7 (see Materials and Methods) disclose a cell line comprising a reporter gene under the control of a BS69 transcription factor

dependent promoter. These documents appear to be novelty destroying for claim 4.

Thus, claims 1, 2, 4, 7-10 do not meet the requirements of Article 33(2) PCT.

2. Inventive step (Article 33(3) PCT)

Claim 6 is not considered to be inventive on the light of D1, which discloses the SEQ ID NO: 2 and a similar method using another sequence.

A cell line comprising a reporter gene under the control of a BS69 transcription factor dependent promoter is already described in D4-D7. Thus, claim 5 is obvious on the light of D4-D7 and is considered not to be inventive.

Claim 3 is considered to be inventive. D2, considered to be the most relevant prior art, fail to describe the relationship between BS69 and Smad proteins. The technical problem to be solved by claim 3 is the provision of methods for identifying modulators of BS69 activity. The solution provided by claim 3 is based on the discovery that the protein BS69 complexes with the Smad 2 and 3 proteins. This solution was neither disclosed nor suggested in the prior art.

3. For the assessment of the present claims 6, 7 and 10 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

## INTERNATIONAL PRELIMINARY

a the contract of the second o

International application No. PCT/GB99/03648

## **EXAMINATION REPORT - SEPARATE SHEET**

#### Re Item VI

#### Certain documents cited

Certain published documents (Rule 70.10)

Application No Patent No

Publication date (day/month/year)

Filing date (day/month/year) Priority date (valid claim) (day/month/year)

WO 99/40220

12.08.1999

04.02.1999

06.02.1998

The intermediate document referred above (= D8) disclose a cell line transformed with a fragment of the PAI-1 promoter. This document, therefore, would appear to disclose or make obvious the subject matter of claim 4.

However, it is considered that the priority of the present application is validly claimed. Should the present application be entered into the regional phase, the above document could be relevant to the question of novelty.

## Re Item VII

## Certain defects in the international application

Reference to prior art being incorporated by reference is not allowed, as the application should be self-contained. Therefore, sentences like "...is incorporated herein by reference" in page 11 line 29 contravene Guidelines C II, 4.17 PCT.

#### Re Item VIII

## Certain observations on the international application

- Claim 1 is not in line with the description (Article 6 PCT), which on page 9, 1. lines 26-31, discloses "a fragment of BS69", but disclaims BRAM1, which can be regarded as a fragment or derivative of BS69.
- Claims 6 and 7 are not clear: it is not possible to make reference in the 2. claims to SEQ ID disclosed in other patent documents, as the application should be self-contained. Therefore, these claims do not meet the requirements of Article 6 PCT.
- Claim 1 contains no technical feature and tries to define the subject-matter in 3. vague and imprecise terms (Article 6 PCT).



<b>1</b>	From the INTERNATIONAL BUREAU		
PCT	То:		
NOTIFICATION OF THE RECORDING OF A CHANGE  (PCT Rule 92bis.1 and Administrative Instructions, Section 422)  Date of mailing (day/month/year) 18 September 2000 (18.09.00)	BILL, Kevin AstraZeneca Global Intellectual Property P.O. Box 272, Mereside Alderley Park Macclesfield, Cheshire SK10 4TG ROYAUME-UNI		
Applicant's or agent's file reference			
PHM 70421/WO	IMPORTANT NOTIFICATION		
International application No. PCT/GB99/03648	International filing date (day/month/year) 04 November 1999 (04.11.99)		
The following indications appeared on record concerning:      X the applicant	the agent the common representative		
Name and Address ASTRAZENECA UK LIMITED 15 Stanhope Gate London W1Y 6LN	State of Nationality  GB  Telephone No.		
United Kingdom	Facsimile No.		
	Teleprinter No.		
The International Bureau hereby notifies the applicant that t     X the person the name the add	dress the nationality the residence		
Name and Address ASTRAZENECA AB	State of Nationality State of Residence SE SE		
S-151 85 Södertälje Sweden	Telephone No.		
	Facsimile No.		
	Teleprinter No.		
3. Further observations, if necessary:			
4. A copy of this notification has been sent to:	=		
X the receiving Office	the designated Offices concerned		
the International Searching Authority	X the elected Offices concerned		
X the International Preliminary Examining Authority	other:		
The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer Christine Carrié		
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38		

٦ From the INTERNATIONAL BUREAU **PCT** NOTIFICATION OF THE RECORDING BILL, Kevin AstraZeneca **OF A CHANGE** Global Intellectual Property P.O. Box 272, Mereside (PCT Rule 92bis.1 and Alderley Park Administrative Instructions, Section 422) Macclesfield, Cheshire SK10 4TG ROYAUME-UNI Date of mailing (day/month/year) 18 September 2000 (18.09.00) Applicant's or agent's file reference **IMPORTANT NOTIFICATION** PHM 70421/WO International application No. International filing date (day/month/year) PCT/GB99/03648 04 November 1999 (04.11.99) 1. The following indications appeared on record concerning: X the agent the applicant the inventor the common representative State of Nationality State of Residence Name and Address BILL, Kevin AstraZeneca UK Limited Telephone No. Global Intellectual Property 01625 512461 Mereside Alderley Park Macclesfield, Cheshire SK10 4TG Facsimile No. 01625 583358 United Kingdom Teleprinter No. 2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning: the person the name the address the nationality the residence State of Nationality State of Residence Name and Address BILL, Kevin AstraZeneca Global Intellectual Property Telephone No. 01625 582828 P.O. Box 272, Mereside Alderley Park Macclesfield, Cheshire SK10 4TG Facsimile No. 01625 583074 United Kingdom Teleprinter No. 3. Further observations, if necessary: 4. A copy of this notification has been sent to:

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland

the International Searching Authority

the International Preliminary Examining Authority

Authorized officer

Christine Carrié

the designated Offices concerned

the elected Offices concerned

Telephone No.: (41-22) 338.83.38

other:

Facsimile No.: (41-22) 740.14.35

the receiving Office

X

1

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#### **NOTIFICATION OF ELECTION**

(PCT Rule 61.2)

#### From the INTERNATIONAL BUREAU

Tο

Assistant Commissioner for Patents United States Patent and Trademark Office Box PCT Washington, D.C.20231 ETATS-UNIS D'AMERIQUE

Date of mailing (day/month/year)
26 June 2000 (26.06.00)

International application No.
PCT/GB99/03648

International filing date (day/month/year)
O4 November 1999 (04.11.99)

Applicant

GREEN, Isabelle et al

1.	The designated Office is hereby notified of its election made:
	X in the demand filed with the International Preliminary Examining Authority on:
	16 May 2000 (16.05.00)
	in a notice effecting later election filed with the International Bureau on:
	<del></del>
2.	The election X was
	was not
	made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).
	·

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland

Authorized officer

S. Mafla

Telephone No.: (41-22) 338.83.38

Facsimile No.: (41-22) 740.14.35



Inte. .ional Application No PCT/GB 99/03648

A. CLASSIFICATION OF SUBJECT MATTER IPC 7 G01N33/50 C12N5/10

C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

#### B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols) LPC 7-601N-C120

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KUROZUMI K ET AL: "BRAMI, a BMP receptor-associated molecule involved in BMP signalling." GENES TO CELLS, (1998 APR) 3 (4) 257-64., XP000870308 cited in the application	
A İ	page 259, paragraph 3 the whole document	
'`		4-7,10
X	WO 97 00323 A (PROLIFIX LTD; VER NL KANKER INST (NL); HATEBOER GUUS (NL); BERNARD) 3 January 1997 (1997-01-03) cited in the application page 13, line 10 - line 12	1,2,8,9
	-/	

X Further documents are listed in the continuation of box C.	Patent family members are listed in annex.
* Special categories of cited documents :	
"A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date  "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)  "O" document referring to an oral disclosure, use, exhibition or other means	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention  "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone  "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled
"P" document published prior to the international filing date but later than the priority date claimed	in the art. "&" document member of the same patent family
Date of the actual completion of the international search-	Date of mailing of the international search report
25 January 2000	04/02/2000
Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nt, Fax: (+31-70) 340-3016	Gundlach, B

Inte .tional Application No PCT/GB 99/03648

C.(Continu	uation) DOCUMENTS CONSIDERED TO BE RELEVANT	PCT/GB 99	7/03048
Category			Relevant to claim No.
X	HATEBOER G ET AL: "BS69, a novel		1,2,8,9
	adenovirus E1A-associated protein that inhibits E1A transactivation." EMBO JOURNAL, (1995 JUL 3) 14 (13) 3159-69. , XP000579801 cited in the application the whole document		1,2,0,9
X	KEETON ET AL: "Identification of regulatory sequences in the type 1 plasminogen activator inhibitor gene responsive to transforming growth factor beta"  JOURNAL OF BIOLOGICAL CHEMISTRY, US, AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, vol. 266, no. 34, page 23048-23052-23052 XP002110964  ISSN: 0021-9258 page 23048, column 2, paragraph 5 -page 23049, column 1, paragraph 2		4
(	DENNLER S ET AL: "Direct binding of Smad3 and Smad4 to critical TGF beta-inducible elements in the promoter of human plasminogen activator inhibitor-type 1 gene"  EMBO JOURNAL,GB,OXFORD UNIVERSITY PRESS, SURREY, vol. 17, no. 11, page 3091-3100 XP002110965  ISSN: 0261-4189 page 3092, paragraph 2		4
	DE CAESTECKER M P ET AL: "Characterization of functional domains within Smad4/DPC4"  JOURNAL OF BIOLOGICAL CHEMISTRY, US, AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, vol. 272, no. 21, page 13690-13696 XP002084021  ISSN: 0021-9258 page 13692, column 2, line 8 -page 13693, column 1, line 6; figure 2B		4
	YINGLING ET AL: "Tumor suppressor Smad4 is a transforming growth factor b-inducible DNA binding protein" MOLECULAR AND CELLULAR BIOLOGY, US, WASHINGTON, DC, vol. 17, no. 12, page 7019-7028-7028 XP002106769  ISSN: 0270-7306 the whole document		4





Inte. onal Application No PCT/GR 99/03648

C.(Continua	ation) DOCUMENTS CONSIDERED TO BE RELEVANT	PCT/GB 99	7/03648
Category	Citation of document, with indication where appropriate, of the relevant passages		Relevant to daim No.
P , X	WO 99 40220 A (GAUTHIER JEAN MICHEL ;GLAXO GROUP LTD (GB)) 12 August 1999 (1999-08-12) page 29		4
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	. Control all to a La consideration of a considerat		

international application No.

PCT/GB 99/03648

Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)
This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:
1. X Claims Nos.:  because they relate to subject matter not required to be searched by this Authority, namely:  Although claims 6 and 7 are directed to a method of treatment of the human/ animal body, the search has been carried out and based on the alleged effects of the compound/composition. Rule 39.1(iv) PCT - Method for treatment of the human or animal body by therapy
Claims Nos.: because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).
Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)
This International Searching Authority found multiple inventions in this international application, as follows:
As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
Remark on Protest
The additional search lees were accompanied by the applicant's protest.
No protest accompanied the payment of additional search fees.

International Application No. PCT/GB 99 \03648

## FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Although claims 6 and 7 are directed to a method of treatment of the human body, the search has been carried out and based on the alleged effects of the used compounds.

Continuation of Box I.1

Rule 39.1(iv) PCT - Method for treatment of the human or animal body by therapy

**(**)

Information on patent family members

Inte ional Application No PCT/GB 99/03648

Patent document cited in search repor	t	Publication date	Patent family member(s)	Publication date
WO 9700323	A	03-01-1997	AU 6230496 A EP 0833924 A US 5985283 A	15-01-1997 08-04-1998 16-11-1999
WO 9940220	Α	12-08-1999	NONE	
	W0 9700323	WO 9700323 A	W0 9700323 A 03-01-1997	W0 9700323 A 03-01-1997 AU 6230496 A EP 0833924 A US 5985283 A





(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference PHM 70421/W0		cation of Transmittal of International Search Report T/ISA/220) as well as, where applicable, item 5 below.
International application No.	International filing date (day/month/ye	ear) (Earliest) Priority Date (day/month/year)
PCT/GB 99/03648	04/11/1999	10/11/1998
Applicant		
ZENECA LIMITED et al.		
This International Search Report has been according to Article 18. A copy is being tra	n prepared by this International Searchi Insmitted to the International Bureau.	ing Authority and is transmitted to the applicant
This International Search Report consists  X It is also accompanied by	of a total of6 sheets a copy of each prior art document cited	
Basis of the report		
With regard to the language, the language in which it was filed, unl	international search was carried out on ess otherwise indicated under this item	the basis of the international application in the
the international search w Authority (Rule 23.1(b)).	as carried out on the basis of a translat	tion of the international application furnished to this
was carried out on the basis of the	e sequence listing :	in the international application, the international search
l <del>                                     </del>	nal application in written form. rnational application in computer reada	able form
	this Authority in written form.	DIE IOIII.
	this Authority in computer readble form	n.
the statement that the sub	•	listing does not go beyond the disclosure in the
the statement that the info furnished	rmation recorded in computer readable	e form is identical to the written sequence listing has been
2. X Certain claims were fou	nd unsearchable (See Box I).	
3. Unity of invention is lact	king (see Box II).	
4. With regard to the title,		
the text is approved as su	bmitted by the applicant.	•
ı <del></del>	hed by this Authority to read as follows:	
METHODS FOR IDENTIFYIN	IG MODULATORS OF BS69 A	CTIVITY
5. With regard to the <b>abstract,</b> X the text is approved as su		Authority as it appears in Box III. The applicant may,
within one month from the	date of mailing of this international sea	arch report, submit comments to this Authority.
6. The figure of the drawings to be publi		
as suggested by the appli		None of the figures.
because the applicant fail		
because this ligure better	characterizes the invention.	



International application No.

PCT/GB 99/03648

Box I	Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)
This Inte	ernational Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:
1. X	Claims Nos.:  because they relate to subject matter not required to be searched by this Authority, namely:  Although claims 6 and 7 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition. Rule 39.1(iv) PCT - Method for treatment of the human or animal body by therapy
2.	Claims Nos.: because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3.	Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).
Box II	Observations where unity of invention is lacking (Continuation of item 2 of first sheet)
This Inte	ernational Searching Authority found multiple inventions in this international application, as follows:
1.	As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2.	As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3.	As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4.	No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
Remark	The additional search fees were accompanied by the applicant's protest.  No protest accompanied the payment of additional search fees.

## FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Although claims 6 and 7 are directed to a method of treatment of the human body, the search has been carried out and based on the alleged effects of the used compounds.

Continuation of Box I.1

Rule 39.1(iv) PCT - Method for treatment of the human or animal body by therapy

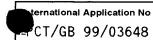
ernational Application No CT/GB 99/03648

A. CLASSIFICATION OF SUBJECT MATTER IPC 7 G01N33/50 C12N ÎPC 7 C12N5/10 C12Q1/68 According to International Patent Classification (IPC) or to both national classification and IPC **B. FIELDS SEARCHED** Minimum documentation searched (classification system followed by classification symbols) G01N C12Q Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practical, search terms used) C. DOCUMENTS CONSIDERED TO BE RELEVANT Category 9 Citation of document, with indication, where appropriate, of the relevant passages Relevant to claim No. X KUROZUMI K ET AL: "BRAM1, a BMP 1-3,8,9receptor-associated molecule involved in BMP signalling." GENES TO CELLS, (1998 APR) 3 (4) 257-64. . XP000870308 cited in the application page 259, paragraph 3 the whole document Α 4-7,10X WO 97 00323 A (PROLIFIX LTD ; VER NL KANKER 1,2,8,9 INST (NL); HATEBOER GUUS (NL); BERNARD) 3 January 1997 (1997-01-03) cited in the application page 13, line 10 - line 12 -/--X Further documents are listed in the continuation of box C. Patent family members are listed in annex. Special categories of cited documents: "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the "A" document defining the general state of the art which is not considered to be of particular relevance invention "E" earlier document but published on or after the international filing date "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such docucitation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or ments, such combination being obvious to a person skilled in the art. "P" document published prior to the international filing date but later than the priority date claimed "&" document member of the same patent family Date of the actual completion of the international search Date of mailing of the international search report 25 January 2000 04/02/2000 Name and mailing address of the ISA Authorized officer European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Gundlach, B Fax: (+31-70) 340-3016

2



		CT/GB 99/03648	
	Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.	
X	HATEBOER G ET AL: "BS69, a novel adenovirus E1A-associated protein that inhibits E1A transactivation." EMBO JOURNAL, (1995 JUL 3) 14 (13) 3159-69., XP000579801 cited in the application the whole document	1,2,8,9	
X	KEETON ET AL: "Identification of regulatory sequences in the type 1 plasminogen activator inhibitor gene responsive to transforming growth factor beta"  JOURNAL OF BIOLOGICAL CHEMISTRY, US, AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, vol. 266, no. 34, page 23048-23052-23052 XP002110964  ISSN: 0021-9258 page 23048, column 2, paragraph 5 -page 23049, column 1, paragraph 2	4	
X	DENNLER S ET AL: "Direct binding of Smad3 and Smad4 to critical TGF beta-inducible elements in the promoter of human plasminogen activator inhibitor-type 1 gene"  EMBO JOURNAL,GB,OXFORD UNIVERSITY PRESS, SURREY, vol. 17, no. 11, page 3091-3100 XP002110965  ISSN: 0261-4189 page 3092, paragraph 2	4	
X	DE CAESTECKER M P ET AL: "Characterization of functional domains within Smad4/DPC4" JOURNAL OF BIOLOGICAL CHEMISTRY,US,AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, vol. 272, no. 21, page 13690-13696 XP002084021 ISSN: 0021-9258 page 13692, column 2, line 8 -page 13693, column 1, line 6; figure 2B		
X	YINGLING ET AL: "Tumor suppressor Smad4 is a transforming growth factor b-inducible DNA binding protein" MOLECULAR AND CELLULAR BIOLOGY,US,WASHINGTON, DC, vol. 17, no. 12, page 7019-7028-7028 XP002106769  ISSN: 0270-7306 the whole document	4	
	-/		



	tion) DOCUMENTS CONSIDERED TO BE RELEVANT		,
ategory °	Citation of document, with indication, where appropriate, of the relevant passages		Relevant to claim No.
' , X	WO 99 40220 A (GAUTHIER JEAN MICHEL ;GLAXO GROUP LTD (GB)) 12 August 1999 (1999-08-12) page 29	-	4
			-
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